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Previews

Pluripotency Network in Embryonic Stem Cells: Maybe Leibniz Was Right All Along

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The transcription factors Tcf3 and Nanog regulate many genes in embryonic stem cells, but according to two reports in this issue of *Cell Stem Cell* (Festuccia et al., 2012, Martello et al., 2012), only one, *Esrrb*, encoding an orphan nuclear hormone receptor, truly matters in the maintenance of self-renewal.

If philosophy is the mother of all sciences, the past century has witnessed an expected adolescent rebellion. Hardly a year goes by without someone claiming that philosophical questions have been crushed under the relentless march of progress. Recent events suggest this hubris might be, well, hubris.

Over the past decade we have struggled to understand how pluripotency is established and maintained, as new studies continue to delineate discrete sets of signaling pathways (including Lif, Fgf-Erk, and Wnt) and critical transcription factors (especially Oct4, Sox2, and Nanog) that are essential for maintaining the pluripotent state (reviewed in Dejosez and Zwaka, 2012). Two papers in this issue of *Cell Stem Cell* (Festuccia et al., 2012, Martello et al., 2012) refine our understanding of the transcriptional control of pluripotency by showing that a single gene may be the only essential target of both Wnt signaling and the transcription factor Nanog. If confirmed, this discovery would provide a new emphasis for embryonic stem cell (ESC) research.

Originally, the Lif pathway (with its downstream effector Stat3) captured the most interest in pluripotency regulation, because Lif was the first factor found to promote self-renewal of mouse ESCs by replacing feeder cells (mouse embryonic fibroblasts necessary for ESC growth). With further investigation, more core constituents of the stem cell signaling machinery emerged: the antineural BMPs, the differentiation-promoting Erk pathway, and the Wnt signaling axis. Wnt signaling attracted special attention not only because of its broad involvement in nearly every other stem (and cancer) cell system but also because it engages the transcriptional effector Tcf3, which is thought to play a critical, nuanced role in regulating pluripotency, either by counteracting or, in some instances, collaborating with other pluripotency factors (Cole et al., 2008; ten Berge et al., 2011; Yi et al., 2011).

With this in mind, Smith and colleagues (Martello et al., 2012) sought to identify critical Tcf3 target genes. They first generated a compendium of such targets through an experimental and intellectual deduction process that pointed to five genes probable to be critically regulated by Tcf3 (*Esrrb*, *Klf2*, *Nanog*, *Nr0b1*, and *Tcfcp2l1*). Further experimentation revealed that only *Esrrb* was necessary for mediating Tcf3's effects on ESC self-renewal. These authors also found that constitutive expression of *Esrrb* can replace Gsk3 inhibition and maintain ESC self-renewal. Several carefully crafted experiments seem to suggest that *Esrrb* functions in parallel with Lif/Stat3. Surprisingly, when Smith and colleagues knocked out or knocked down *Esrrb*, they discovered that this gene was almost entirely dispensable for ESC self-renewal, but only in a Lif-dependent manner. They explained this result by arguing that *Esrrb* appears to have significant functional redundancy with other pluripotency factors.

Chambers and colleagues (Festuccia et al., 2012) took a slightly different experimental route to address the same issue. Nanog is considered to be essential for establishing pluripotency, and experimentally enforced expression can compensate for the loss of Lif signaling in ESCs. Nanog has been reported to bind over 5,000 genes, but Chambers and colleagues were able to narrow this rather broad field to the relevant targets by examining the behavior of these targets in response to different Nanog levels. As in Martello et al., only *Esrrb* appeared to be a functionally relevant target. Indeed, its expression could ensure self-renewal even in the absence of Nanog or Lif (*Nanog*$^{-/-}$ and *Lif*$^{-/-}$ cells). Moreover, one of the defining features of Nanog is its capacity to propel so-called epiblast stem cells (EpiSCs, another pluripotent stem cell caught in a developmentally more advanced stage) into "full-blown" pluripotentiality. Esrrb performed in this context equally well, despite the genetic absence of *Nanog*. To extend the functional analogy between Nanog and Esrrb even further, the authors demonstrate that, as with Nanog, Esrrb can push neural stem cells and so-called partially reprogrammed iPSCs out of their gray zones (where they acquired many essential features of pluripotency yet lack the fully activated machinery of this state). Finally, as with Smith's group, Chambers and colleagues found that Esrrb is dispensable for self-renewal, ostensibly because of significant overlap among the other canonical pluripotency factors.

So, what is special about Esrrb, and does it matter that both Tcf3 and Nanog converge on this locus to sustain ESC self-renewal? Molecularly, Esrrb belongs to the superfamily of nuclear hormone receptors. Even though the mouse knockout of *Esrrb* (placental defects but no loss of pluripotency in the early embryo) and its relatively broad expression pattern do not suggest a major role in pluripotency, this transcription factor has been extensively linked to Oct4, Sox2, and Nanog and therefore is considered a bona fide member of the pluripotency protein club (Ivanova et al., 2006; van den Berg et al., 2010; Zhang et al., 2008). The specific role of Esrrb

remains murky, however, especially when one considers that it binds rather promiscuously to the majority of genes expressed in ESCs and interacts indiscriminately with the basic transcriptional machinery.

How, then, does one account for the seemingly unnecessarily redundant and complex relationship among Esrrb, Nanog, and Tcf3? It may be that Esrrb cannot be explained by our usual cause-and-effect framework of transcriptional control. That is, if transcriptional systems emerged at different times in evolution, as most data indicate, but in some cases were retained regardless of the organism's shifts in functional needs, Esrrb might well represent a "molecular appendix"—we still have it and cannot get rid of it because it continues to perform a (minor) function (Figure 1A). An evolutionary basis for the observed relationship between Esrrb and the rest of the pluripotency machinery is further suggested by very solid evidence for extensive rewiring of transcriptional networks (possibly via transposon/retrotransposon-mediated activities) (Kunarso et al., 2010) that may have involved the superfamily of nuclear receptors, Esrrb in particular.

These two reports (Festuccia et al., 2012, Martello et al., 2012), together with previous accounts of the transcriptional regulation of ESC pluripotency (Dejosez and Zwaka, 2012), raise the intriguing possibility that coordination among essential transcription factors in ESCs comes about not through causal linkages but as the result of a preset, almost Leibnizian harmony that synchronizes the activities of these factors without encroaching on their independence. Gottfried Leibniz (1646–1716), known as the last "universal genius," rejected a strictly materialistic metaphysics and postulated that reality was formed of monads, simple substances that perceive the state of all other monads, exist in a specific state, and are capable of changing that state. (Alfred North Whitehead later postulated a similar metaphysics that emphasized process over material substance.) Thus, Esrrb and its companions in ESCs may represent "transcriptional monads" capable of constantly fine-tuning the entire pluripotency network at any moment. In this system Esrrb would be highly responsive to direct inputs from Tcf3 or Nanog but would act only in accord with the status of all other transcriptional monads involved in the maintenance of pluripotency (Figure 1B). Thus, even if we continue to uncover so-called core regulatory elements in ESCs, it may not be possible to establish a true transcriptional control network until we begin to question the prevailing hierarchical model of gene transcription. To fully comprehend the experimental measurements reported by Festuccia et al. and Martello et al., for example, it may be necessary to develop novel mathematical models of transcriptional control based on nonlinear computation of transcriptional states.

Perhaps philosophy has not relinquished her dominion over science just yet.

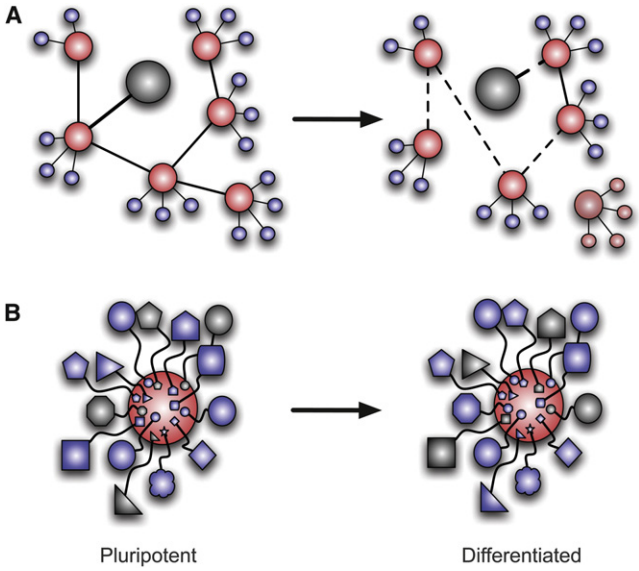

Figure 1. Different Models Explaining the Apparently Excessive Redundancy and Complexity of Interactions among Pluripotency Factors in ESCs

(A) Transcriptional system that emerged at some point during evolution (left). Retention of certain components of this system led to extensive rewiring of present-day transcriptional circuits responsible for pluripotency (right, dashed lines).

(B) Array of proposed "transcriptional monads" capable of constantly fine-tuning the entire pluripotency network at any moment through harmonic connections. An individual monad (red) acts only in accord with the status of all other transcriptional monads (blue, gray) to determine stem cell states and transitions (e.g., differentiation).

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