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Perspective

A Precarious Balance: Pluripotency Factors as Lineage Specifiers

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Understanding the basis of the unrestricted multilineage differentiation potential of pluripotent cells will be of developmental and translational consequence. We propose that pluripotency transcription factors are lineage specifiers that direct commitment to specific fetal lineages. Individual factors bestow the ability to differentiate into particular cell types, and concomitant expression of multiple lineage specifiers within pluripotent cells enables differentiation into every fetal lineage. Moreover, we speculate that, rather than being an intrinsically stable "ground state," pluripotency is an inherently precarious condition in which rival lineage specifiers continually compete to specify differentiation along mutually exclusive lineages.

Pluripotent cells, such as mouse embryonic stem cells (ESCs) and their embryological antecedents—the cells of the periimplantation epiblast—have the capacity to differentiate into any cell type present within the fetus. Hence, pluripotent cells exist at the summit of the proverbial mountain of developmental potential (as illustrated by Graf and Enver, 2009). How such a vast range of lineage choices is made available remains cryptic, however. We herein articulate a speculative basis for the unrestricted multilineage differentiation potential of pluripotent cells, founded on a reinterpretation of existing findings. Instead of imagining pluripotency as an intrinsically stable "ground state" (Jaenisch and Young, 2008; Silva and Smith, 2008; Wray et al., 2010; Young, 2011), we envisage that it is inherently unstable and that pluripotency is defined by transcriptional competition between the lineage-specifying actions of pluripotency factors.

Pluripotency Factors Are Lineage Specifiers

The pluripotent state is supervised by a regime of transcription factors that endow ESCs with their salient characteristics—as Silva and Smith have summarized aptly, "transcription factors rule pluripotency" (Silva and Smith, 2008). Loss of individual pluripotency transcription factors frequently prompts ESC differentiation to specific lineages (reviewed by Lessard and Crabtree, 2010).

Based on such findings, the current model of pluripotency proposes that individual pluripotency factors act to prohibit ESC cell differentiation along specific lineages (Figure 1A) and that, as a result, ESCs are ensconced by a shield of protective transcription factors that collaboratively inhibit differentiation to all lineages in order to preserve an undifferentiated state (Jaenisch and Young, 2008; Silva and Smith, 2008; Young, 2011). This model accounts for how ESCs can remain undifferentiated but provides little explanation for their multilineage differentiation potential. Indeed, if pluripotency factors redundantly upregulate one anothers' expression while constitutively inhibiting differentiation (Jaenisch and Young, 2008; Silva and Smith, 2008; Young, 2011), it would in fact seem difficult for ESCs to ever differentiate and commit to any subordinate lineage.

One of the fundamental predictions of this prevailing hypothesis is that overexpression of individual pluripotency factors should prohibit ESC differentiation. However, it has been repeatedly found that overexpression of pluripotency factors in ESCs often induces differentiation. For example, overexpression of the classical pluripotency factor *Oct4* specifies mesodermal differentiation (Niwa et al., 2000); *Sox2* overexpression prompts neuroectodermal specification (Kopp et al., 2008); overexpression of *Esrrb*, *Sall4*, or *Tbx3* elicits endodermal determination (Ivanova et al., 2006; Lu et al., 2011; Zhang et al., 2006); and overexpression of *Dax1* directs trophectodermal respecification (Sun et al., 2009). Moreover, *Nanog* overexpression in human ESCs directs mesendodermal differentiation (Teo et al., 2011; Yu et al., 2011), and *Sip1* overexpression begets neuroectodermal commitment (Chng et al., 2010). Recent high-throughput studies have also identified additional pluripotency factors that similarly provoke ESC differentiation when overexpressed (Ivanova et al., 2006; Nishiyama et al., 2009).

These findings that pluripotency factor overexpression frequently prompts ESC differentiation are difficult to reconcile with previous assertions that pluripotency factors constitutively suppress lineage commitment (Jaenisch and Young, 2008; Silva and Smith, 2008; Young, 2011). To overcome such difficulties, we would like to suggest a complementary hypothesis for the consideration of the field.

We propose that many pluripotency factors function as classical lineage specification factors (reviewed by Enver and Greaves, 1998), directing ESC differentiation to a specific fetal lineage while prohibiting commitment to mutually exclusive lineages. Thus, we envisage that pluripotency is not maintained by a regime of inhibitory transcription factors that cooperatively block differentiation to all lineages. Instead, we propose an alternative scenario in which many individual pluripotency factors are continually attempting to specify ESC differentiation to their own lineage of interest (Figure 1A).

Furthermore, we venture that the ability of ESCs to differentiate into specific lineages derives from their intrinsic lineage specifiers, each of which provides ESCs with the ability to

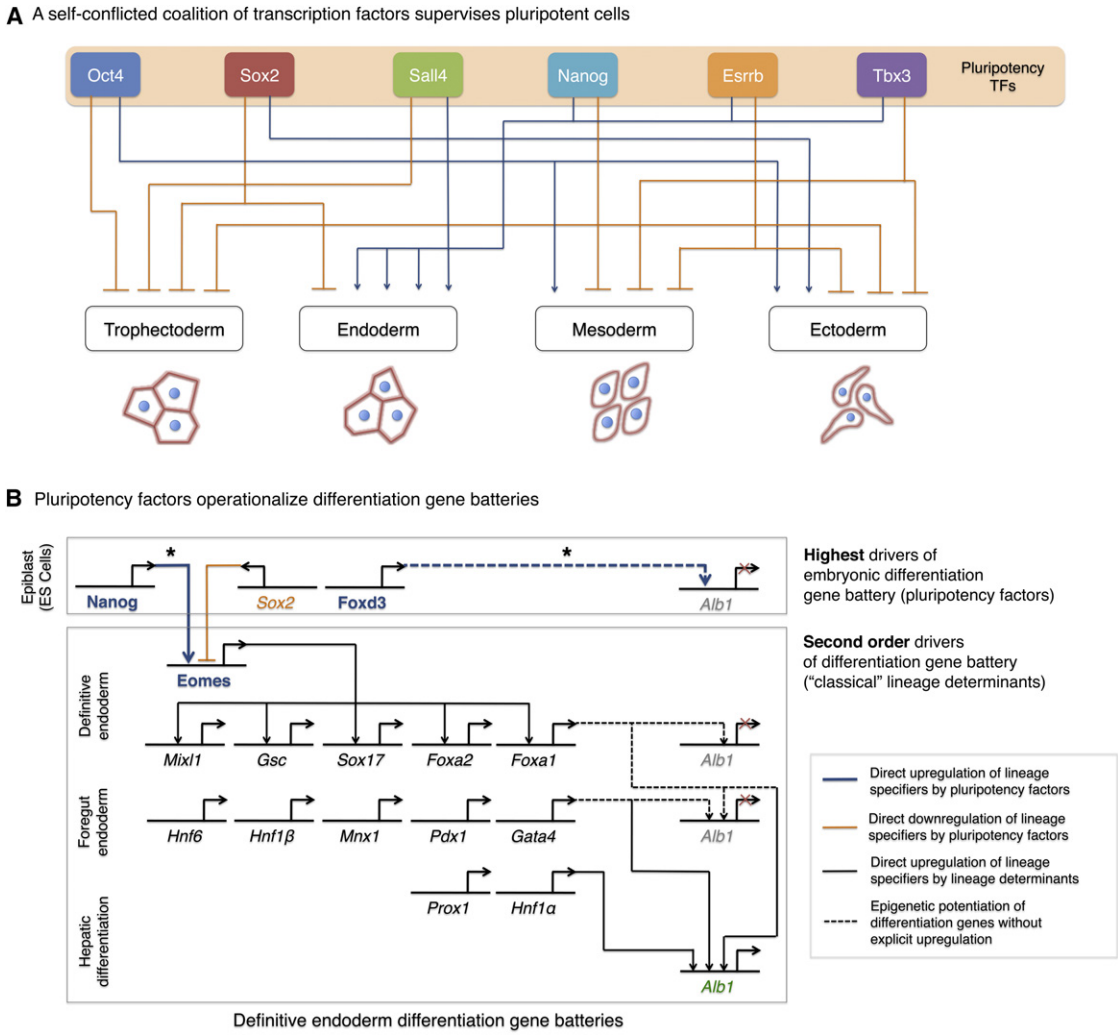

Figure 1. The Transcriptional Agency that Oversees Pluripotency

(A) A divided coalition of competing transcription factors oversees pluripotency. Pluripotency transcription factors exert lineage-specific blockades on differentiation to particular lineages (hatched orange lines) while often concomitantly directing differentiation to an alternative lineage (blue arrows). Hence, pluripotency factors function as classical lineage specifiers, and confer ESCs with the ability to differentiate to specific fetal lineages—thus providing a basis for the multilineage differentiation potential of pluripotent cells. Coincident expression of diverse lineage specifiers within undifferentiated ESCs leads these factors to cross-inhibit one another, resulting in no net commitment to any particular lineage. Nevertheless, slight perturbations in the expression of any transcription factor would result in collapse of this fragile transcriptional equilibrium and consequential lineage commitment—hence, pluripotency is inherently transcriptionally insecure. TF, transcription factors.

(B) Pluripotency factors direct the lineage specification of ESCs by upregulating lineage determinants. Pluripotency factors activate differentiation gene batteries by potentiating expression of both master drivers and terminal differentiation effectors (Davidson, 2010). Here, the definitive endoderm differentiation gene battery is shown, reconstructed from data taken from human ESCs, mouse ESCs, and mouse development. Nanog upregulates *Eomes*, one of the master drivers of the definitive endoderm differentiation gene battery, while Foxd3 concomitantly binds to *albumin* (*Alb1*) and keeps it free of DNA methylation and ready for expression. As the epiblast segues to definitive endoderm, Foxa1 and Gata4 replace Foxd3 in potentiating *Alb1* expression, and as hepatic differentiation occurs, accessory transcription factors (e.g., Hnf1α) accumulate on *Alb1*, finally leading to its expression. Asterisks denote epigenetic potentiation of differentiation genes by pluripotency factors in ESCs.

differentiate into one or more particular fetal lineages. For example, Oct4 confers ESCs with the capability to differentiate into mesoderm (Zeineddine et al., 2006), whereas Nanog provides the ability to differentiate into definitive endoderm (Teo et al., 2011). In our model, the expression of a myriad of diverse lineage specifiers within ESCs provides them with all the developmental regulators necessary to differentiate toward any major fetal lineage.

Our paradigm also offers an explanation for why tissue-specific differentiation factors such as *Sox2* and *Zic3* are expressed in and are important for ESCs, which would be difficult to rationalize a priori. We propose that these lineage determinants reprise some of their roles in fetal development within pluripotent cells in order to enable ESCs to differentiate towards specific fetal lineages.

Lineage Specification by Pluripotency Factors: Testing the Model

As discussed above, previous investigations have found that that many pluripotency factors have explicit lineage specifying

activities. However, additional experimentation will be needed to reify our proposed model.

In particular, while exogenous pluripotency factor overexpression frequently elicits ESC differentiation to specific lineages, it is important to ascertain whether these factors are endogenously required for ESCs to differentiate into these cell types. One intuitive experiment is to knock down individual lineage-specifying pluripotency factors within ESCs and then test whether these cells can still differentiate into the relevant lineages.

This type of experiment has already been performed for some factors. Knockdown of mesodermal determinant *Oct4* immediately before differentiation renders ESCs largely incapable of mesodermal differentiation (Zeineddine et al., 2006). Likewise, knockdown of the primitive endoderm sponsor *Tbx3* abrogates the ability of mouse ESCs to differentiate into primitive endoderm (Lu et al., 2011), and loss of the endodermal specifier *Nanog* in human ESCs compromises their capacity for mesendodermal differentiation (Teo et al., 2011). From such findings, we surmise that *Oct4*, *Nanog*, and *Tbx3* represent bona fide lineage specifiers that confer ESCs with the ability to differentiate into specific embryonic lineages.

It will also be illuminating to test whether pluripotency factors operate as lineage specifiers in vivo within their native epiblast context (not just within ESCs in vitro). This seems to be a likely contingency because around the time of gastrulation, *Oct4*, *Sox2*, and *Nanog* are no longer expressed throughout the entire epiblast, but instead, they are only expressed by select subpopulations of cells that are already fated to differentiate into certain germ layers (reviewed by Teo et al., 2011). Such lineage-restricted expression raises the formal possibility that individual pluripotency factors may specify epiblast differentiation to particular germ layers. Indeed, *Oct4* is upregulated during mesodermal commitment of the epiblast—and conspicuously, if *Oct4* is knocked down, postimplantation epiblast cells are largely incapable of mesodermal differentiation (Zeineddine et al., 2006).

The construction of inducible pluripotency factor-knockout embryos would allow for rigorous evaluation of our model by examining whether temporally controlled ablation of pluripotency factors in the postimplantation epiblast precludes differentiation to specific fetal lineages. We also predict that in vivo overexpression of pluripotency factors such as *Oct4* and *Sox2* within the early postimplantation epiblast would induce all epiblast cells to adopt a mesodermal fate or a neuroectodermal fate, respectively, overcoming natural developmental assignment of epiblast cells to diverse germ layer fates.

Mechanisms Underlying Lineage Specification

Intuitively, if pluripotency factors function as lineage specifiers, they must bind to and activate the expression of genetic loci encoding differentiation genes. Consistent with this idea, pluripotency transcription factors are widely known to bind to differentiation genes within undifferentiated ESCs (reviewed by Jaenisch and Young, 2008; Young, 2011). Such interactions have been previously interpreted to be repressive (Jaenisch and Young, 2008; Young, 2011), pursuant to the prevailing model that pluripotency factors constitutively inhibit differentiation. However, finding that a pluripotency factor is bound to the promoter of a differentiation gene through chromatin immunoprecipitation (chIP) does not ipso facto mean that it is repressing its target.

Conversely, when chIP-predicted interactions between pluripotency factors and differentiation genes were interrogated at a functional level, it was found that some pluripotency factors actually potentiate the expression of certain of the lineage specification genes that they bind (Figure 1B). For example, Nanog provides human ESCs with the ability to differentiate into definitive endoderm by binding to and directly upregulating the expression of *Eomes*, one of the master drivers of definitive endoderm differentiation (Teo et al., 2011). Likewise, Tbx3 endows mouse ESCs with the capability to differentiate into primitive endoderm by directly binding to the *Gata6* promoter and displacing the PRC2 H3K27 methyltransferase complex, thus liberating the endodermal specification gene *Gata6* from repressive H3K27 methylation (Lu et al., 2011).

Pluripotency transcription factors can also help to presage later differentiation events. For example, Foxd3 binds to the liver-specific *albumin* enhancer (in mouse ESCs) and keeps it free of DNA methylation, such that *albumin* may later be expressed in hepatic cells after endodermal determination (Xu et al., 2009). Similarly, Sox2 targets *immunoglobulin lambda* for activatory H3K4 dimethylation within ESCs, such that it may later be expressed in pro-B lymphocytes after hematopoietic determination (Liber et al., 2010).

Epigenetic potentiation of differentiation genes by pluripotency factors is difficult to rationalize within the framework of the existing model (Silva and Smith, 2008). However, with our present model, it may be readily understood a posteriori that that these pluripotency factors are simply carrying out their lineage specification activities and are attempting to upregulate their downstream lineage-specific genes in order to effect differentiation.

Overall, we suggest that the initial differentiation of ESCs does not require the activation of some dormant dedicated lineage specifier that then contends with resident pluripotency factors to transact differentiation, as previously proposed (Silva and Smith, 2008). Instead, we aver that pluripotency factors are themselves dominant lineage specifiers that actively upregulate specific lineage determinants in undifferentiated ESCs to maintain a diverse range of lineage commitment options and to continually provide opportunities for differentiation. ESCs already express all the transcription factors required to engage any major fetal lineage differentiation program and have in hand all the necessary transcription factors needed to initially differentiate into any primary fetal germ layer.

Hence, when considering the hierarchical differentiation gene batteries involved in the specification of embryonic lineages (reviewed by Davidson, 2010), we assert that pluripotency factors may be regarded as the highest-level upstream drivers of any fetal differentiation gene battery (Figure 1B).

Extrinsic Signaling Is Required to Maintain Pluripotency

Superficially, our assignment of pluripotency factors as lineage specifiers might seem at odds with the fact that ESCs expressing these transcription factors can be perennially maintained in an undifferentiated state. However, this quandary may be readily resolved when one considers that an inherent property of lineage specification factors is the ability to suppress commitment to

mutually exclusive lineage options (reviewed by Enver and Greaves, 1998). Diverse lineage specifiers associated with mutually exclusive lineages are expressed concomitantly in ESCs, and thus, they must cross-inhibit one anothers' differentiation-inducing activities (Figure 1A). The end result is temporary prevention of commitment to any major fetal lineage and retention of a net undifferentiated state despite the coexpression of many lineage specifiers. Thus, we propose that undifferentiated self-renewal is maintained by a carefully balanced equilibrium of transcription factors wherein the lineage specifying activities of individual factors are all counteracted by one another.

We speculate that pluripotency is an intrinsically unstable state of affairs, as differentiation can be readily elicited by the stochastic upregulation or downregulation of individual pluripotency factors. Upregulation of particular transcription factors could directly specify lineage commitment (à la Oct4). Conversely, downregulation of any pluripotency factor would release its lineage-specific blockade on differentiation, enabling lineage specifiers directing differentiation to these previously repressed lineages to proceed unopposed. Such predictions appear to be substantiated by salient findings that subtle fluctuations in *Oct4* or *Sox2* expression elicit differentiation (Kopp et al., 2008; Niwa et al., 2000). Thus, we predict that in order for ESCs to remain undifferentiated, the expression levels of key pluripotency factors must be carefully controlled to ensure that no single factor's lineage specifying activities become dominant.

Extrinsic cytokine signaling provides a mechanism through which the expression of various pluripotency factors could be providently controlled (Figure 2A). For example, in mouse ESCs, LIF signaling directly upregulates expression of transcription factors *Klf4* and *Tbx3* and indirectly upregulates expression of *Sox2* and *Nanog* (Niwa et al., 2009), TGFβ signaling directly upregulates *Oct4* expression (Zeineddine et al., 2006), and BMP signaling upregulates *Id* transcription factors that oppose neuroectodermal differentiation (Ying et al., 2003), thus restraining neuroectodermal specifiers such as Sox2. Continual signaling through these pathways could engineer undifferentiated transcriptional states in which opposing pluripotency factors are expressed at comparable levels. Thus, we conjecture that undifferentiated ESC self-renewal may only be enduringly maintained through continual extrinsic signaling.

The Fallibility of Extrinsic Signaling

However, one may deduce that it would be inherently inefficient and error-prone to continually maintain undifferentiated states through the perpetual re-upregulation of opposing pluripotency factors via extrinsic signaling. Individual cells often respond heterogeneously, asynchronously, or sometimes not at all to extrinsic signals. Upon receiving an extrinsic signal, many cells fail to engage the subordinate signal transduction pathway and express assigned target genes (Fiering et al., 1990; Tay et al., 2010). If pluripotency factor expression is acutely contingent upon extrinsic signaling, then heterogeneous transduction of extrinsic signals by ESCs should generate correspondingly heterogeneous expression of pluripotency factors among individual ESCs (Figure 2B). Indeed, although *Klf4* and *Tbx3* are direct transcriptional targets of LIF signaling, they are not even expressed by all ESCs (Niwa et al., 2009). Through re-examination of published data sets (Tang et al., 2010), we have found a previously unrecognized 10-fold variation in both *Oct4* and *Sox2* expression (Figure 2B) between individual ESCs. Other pluripotency factors have also been reported to be heterogeneously expressed in ESC cultures (reviewed by Graf and Stadtfeld, 2008).

Variable expression of pluripotency factors with lineage specifying activities seems potentially problematic, especially in the case of *Oct4* and *Sox2*, given that only 2-fold variations in their expression levels are sufficient to elicit differentiation (Kopp et al., 2008; Niwa et al., 2000). Should our model hold true, we predict that ESCs expressing exceedingly high levels of *Oct4* would be strongly biased toward mesodermal specification, and that those with upregulated *Sox2* would be predisposed to neuroectodermal commitment.

Close Encounters of the Differentiated Kind

Consistent with the lineage-specifying activities of pluripotency factors, significant proportions of ESCs express multifarious genes associated with commitment to diverse lineages, such as the mesodermal specifiers *Brachyury* and *Hes1* (Kobayashi et al., 2009; Suzuki et al., 2006) and the primitive endodermal determinants *Hhex* and *Rest* (Canham et al., 2010; Yamada et al., 2010). Individual ESCs expressing *Brachyury*, *Hes1*, or *Hhex* are significantly predisposed to differentiate into mesoderm or primitive endoderm, respectively (Suzuki et al., 2006; Kobayashi et al., 2009; Canham et al., 2010). Fluctuating expression of lineage specifiers in undifferentiated ESCs probably underlies the spontaneous differentiation of ESCs at appreciable frequencies even under optimal culture conditions (Smith, 2001; Smith et al., 1992). What remains to be done is to correlate upregulation of lineage-specifying pluripotency factors (e.g., *Oct4*) in single ESCs with consequential upregulation of lineage determinants (e.g., *Brachyury*).

Moreover, it appears that individual ESCs are continually moving to and fro between specific lineage options, as they may flexibly upregulate and then downregulate lineage specifiers such as *Brachyury*, losing associated differentiation biases in the process (Suzuki et al., 2006). It will be insightful to discern the mechanisms that underlie the continual flux of ESCs between unbiased and lineage-inclined states.

We conclude that spontaneous ESC differentiation in steady-state culture conditions again reiterates the transcriptional insecurity of pluripotency and is reflective of an intrinsic inclination of ESCs toward lineage commitment. Spontaneous differentiation is also consistent with a model in which imprecise control of pluripotency factor expression by extrinsic signals generates cell-to-cell transcriptional and functional heterogeneity within ESC cultures.

Can Pluripotency Be Maintained without Extrinsic Intervention?

Is pluripotency an unstable state of exception that may only be maintained through continual extrinsic signaling (as we propose), or is it an intrinsically transcriptionally stable "ground state" that will indefinitely self-maintain in the absence of all extrinsic signals (Wray et al., 2010)?

From a physiological perspective, it is often argued that pluripotency must be intrinsically stable in vivo, as presumptive epiblast cells can be maintained in a pluripotent state for several

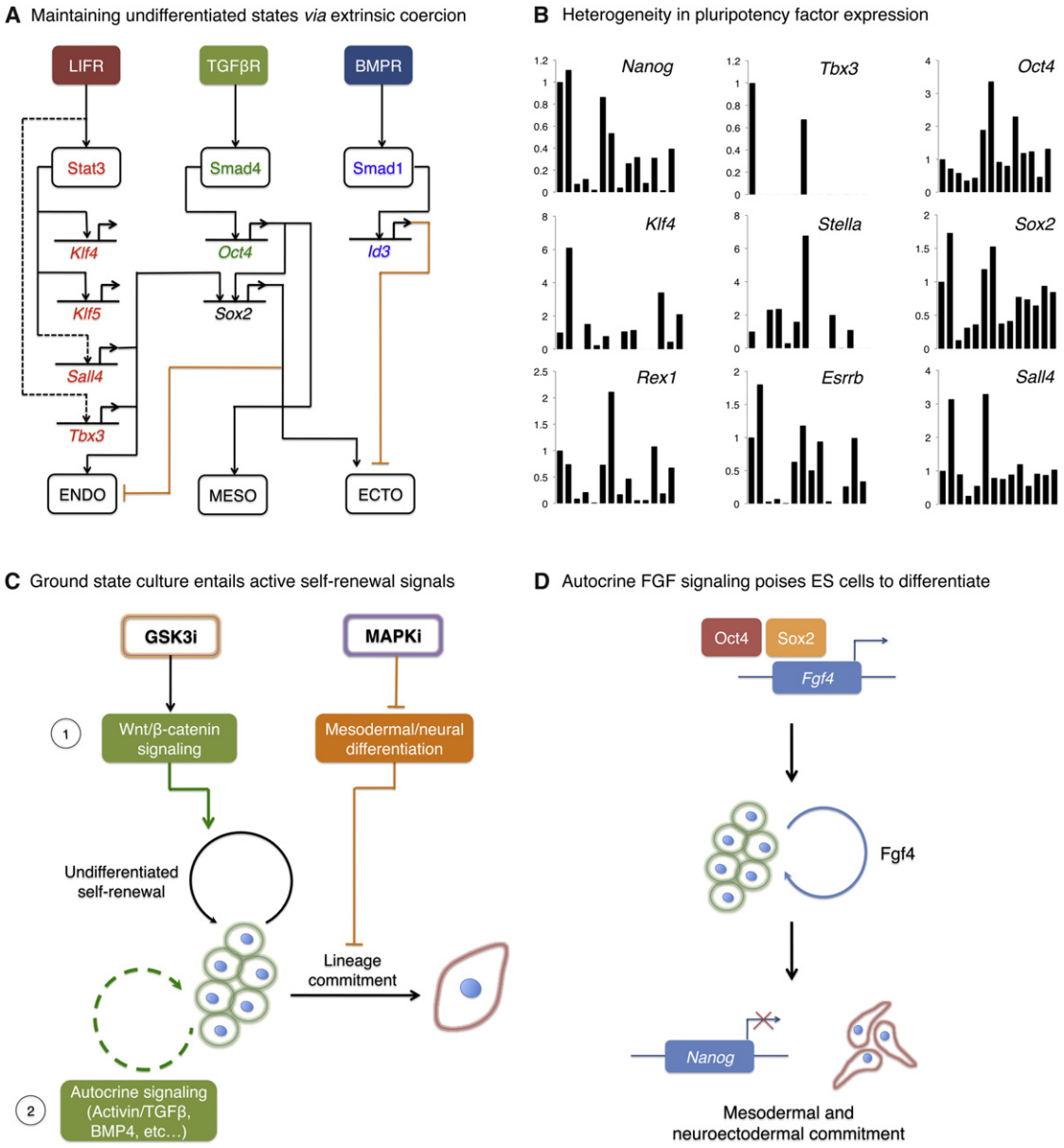

Figure 2. The Mechanisms and Consequences of Maintaining Pluripotency via Extrinsic Signaling

(A) Extrinsic signals attempt to maintain consistent expression levels of pluripotency factors in order to maintain undifferentiated self-renewal. We depict here how LIF signaling, TGFβ signaling, and BMP signaling directly upregulate select pluripotency factors (Niwa et al., 2009; Ying et al., 2003; Zeineddine et al., 2006) via their respective pathway-specific terminal transcriptional effectors in mouse ESCs. These pluripotency transcription factors subsequently cross-regulate one anothers' expression, promote installation of favored lineage-specification programs, and prohibit adoption of mutually exclusive lineage specification programs. Transcriptional cross-regulation of pluripotency factors is partially based on chromatin immunoprecipitation (chIP) data and is thus necessarily tentative. ENDO, MESO, and ECTO represent the endodermal, mesodermal, and ectodermal differentiation gene batteries, respectively. Dashed lines represent STAT3-independent regulation of *Tbx3* by LIF signaling (Niwa et al., 2009) and indirect regulation of *Sall4* by STAT3. Hatched orange lines represent inhibition of neuroectodermal differentiation by Id3 (Ying et al., 2003) and inhibition of endodermal differentiation by Sox2 (Ivanova et al., 2006).

(B) Individual ESCs display heterogeneous expression of the transcription factors *Oct4*, *Sox2*, *Nanog*, *Tbx3*, *Klf4*, *Rex1*, *Stella*, and *Esrrb*. The graphs represent our analysis of published high-throughput single-cell qPCR results on 14 individual ESCs (Tang et al., 2010), initially normalized to *actb* expression and then later normalized to one specific ESC ("ES11").

(C) Undifferentiated self-renewal under "2i" conditions entails mouse ESC culture with a MAPK inhibitor (MAPKi; PD0325901) and a GSK3 inhibitor (GSK3i; CHIR99021). Such "2i" culture conditions are believed to preserve undifferentiated ESCs solely by blockade of differentiation signals (Ying et al., 2008). However, we believe that "2i" culture conditions still entail active self-renewal signals that direct the upregulation of pluripotency factors. Namely, GSK3 inhibition activates Wnt/β-catenin signaling and autocrine self-renewal signals innately produced by ESCs still persist.

(D) Undifferentiated ESCs have an inherent proclivity to differentiate, as Oct4 and Sox2 continually upregulate *Fgf4* expression, which functions as an autocrine signal to downregulate *Nanog* and direct either neuroectodermal or mesodermal commitment (Kunath et al., 2007).

weeks during diapause. However, the prolonged pluripotency exhibited by the epiblast during diapause is strictly dependent on LIF signaling (Nichols et al., 2001). Thus, the extended maintenance of the epiblast's pluripotency observed during diapause is the consequence of extrinsic cytokine signaling (similar to what self-renewing ESCs experience in vitro) and therefore does not necessarily reflect an innate transcriptional stability of the pluripotent condition.

More recent assertions that pluripotency is intrinsically stable are based on the inception of "2i" culture conditions (Ying et al., 2008). In brief, it was found that mouse ESCs could be kept perennially undifferentiated in the absence of any exogenous cytokines if they were treated with two chemical "signal inhibitors"—a MAPK inhibitor and an GSK3 inhibitor—the "2i" culture regimen (Ying et al., 2008). Such "2i" conditions were thought to demonstrate that when ESCs were made bereft of all extrinsic signals (achieved by withdrawal of exogenous cytokines such as LIF and concomitant addition of "signal inhibitors"), they would remain pluripotent; thus, undifferentiated self-renewal was proposed to be the ESC transcriptional "ground state" in the absence of all extrinsic instructions (Silva and Smith, 2008; Wray et al., 2010; Ying et al., 2008).

We would argue that this conclusion is somewhat premature. "2i" culture conditions do not provide an entirely signal-free environ for ESC self-renewal. The GSK3 inhibitor is not entirely a "signal inhibitor," but rather, it directly activates Wnt/β-catenin signaling (Figure 2C), a dominant ESC self-renewal pathway (Sato et al., 2004)—and in fact, the GSK3 inhibitor can be functionally replaced by a direct activator of Wnt/β-catenin signaling, Wnt3a (Ying et al., 2008). Moreover, ESCs autonomously produce several cytokines, including Activin/TGFβ and BMP4, that support their own undifferentiated self-renewal in autocrine fashion (Ogawa et al., 2007; Ying et al., 2003). These autocrine ESC cytokines could still autonomously direct self-renewal even in the absence of exogenous cytokines in "2i" culture conditions (Figure 2C). In short, we believe that "2i" culture conditions maintain undifferentiated self-renewal in the absence of exogenous cytokines by alternatively transmitting active self-renewal signals through the Wnt/β-catenin pathway and autocrine signaling loops. One may readily assess our hypothesis by testing whether "2i" conditions can maintain *β-catenin*$^{-/-}$ ESCs.

It will be inherently difficult to ever demonstrate definitively that ESCs constitutively self-renew in the absence of extrinsic signals, given the multitudinous signaling pathways that would need to be silenced to truly monitor pluripotent cell behavior bereft of all external cues. Our prediction is that suppression of differentiation signals alone is not sufficient to maintain ESC self-renewal. Rather, we argue that some form of direct extrinsic intervention is always required to continually reinforce the unstable ESC transcription factor regime and prevent its collapse at the hands of its resident lineage specifiers.

Pluripotency, the Precarious Potential

In sum, we propose that within pluripotent cells there exists a state of continual conflict between pluripotency transcription factors that seek to direct ESC differentiation to opposing lineages. Austin Smith previously coined the phrase "the battlefield of pluripotency" (Smith, 2005), and his descriptor elegantly illustrates our present proposal. We suggest that pluripotency is inherently ephemeral, as even when ESCs are undifferentiated, they continually produce the autocrine differentiation signal FGF4 in order to destabilize themselves (see Kunath et al., 2007 and Figure 2D), and their intrinsic lineage specifiers are vying for dominance among one another in order to instruct commitment to different lineages. We propose that extrinsic signals must be continually applied in order to sustain undifferentiated self-renewal and to ensure that no lineage specifying pluripotency factor becomes dominant.

The instability of pluripotency that we have proposed here is compatible with the physiological raison d'être of pluripotency. In the early embryo, epiblast cells transiently ascend to pluripotency in order to attain the ability to differentiate into all fetal cell types. Then, their pluripotency is expended shortly thereafter within several days to generate all the cells that will populate the fetus. There is no physiological need for pluripotent cells to continually persist throughout development or adulthood. Thus, we believe that no molecular provisions have been made to ensure that pluripotent cells are capable of long-term independent self-maintenance.

We conclude by suggesting a fundamental revision to how the functions of many pluripotency transcription factors are typically perceived. Given the extraordinary catalog of lineage commitment options available to pluripotent cells, it is unclear why the present focus on the function of pluripotency transcription factors is on how they curtail lineage commitment (Jaenisch and Young, 2008; Silva and Smith, 2008; Young, 2011). A complementary appreciation for the lineage specifying activities of pluripotency factors may help provide an understanding of what underlies the remarkable multilineage differentiation potential of pluripotent cells.

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