

# Naive and Primed Pluripotent States

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**After maternal predetermination gives way to zygotic regulation, a ground state is established within the mammalian embryo. This tabula rasa for embryogenesis is present only transiently in the preimplantation epiblast. Here, we consider how unrestricted cells are first generated and then prepared for lineage commitment. We propose that two phases of pluripotency can be defined: naive and primed. This distinction extends to pluripotent stem cells derived from embryos or by molecular reprogramming *ex vivo*.**

Mammalian embryos produce extraembryonic cells prior to defining the founder population for the embryo proper (Gardner, 1983; Selwood and Johnson, 2006). The primary role of the extraembryonic lineages is to mediate uterine implantation and subsequent maternal sustenance of the growing embryo and fetus. In recent years, it has been discovered that extraembryonic tissues also supply powerful inductive signals that specify and pattern early development (Beddington and Robertson, 1999). To form the embryo, a pool of uncommitted cells must be established and poised to respond to those signals. This population is the epiblast. Generation of naive epiblast several days after fertilization is a process unique to the mammalian life cycle. This progression does not occur through simple inheritance from the fertilized egg, nor is epiblast specified by segregation of maternal determinants. How the emerging epiblast is shielded from extraembryonic differentiation and concomitantly gains the capacity to generate all cell types of the fetus and subsequent adult, including the germ cells, are fundamental questions in mammalian development. Moreover, the aspiration to exploit *ex vivo* pluripotent stem cells for biomedical benefit surely requires elucidation of their precise nature and relationship to pluripotent cells in the embryo.

## The Limitation of Totipotency

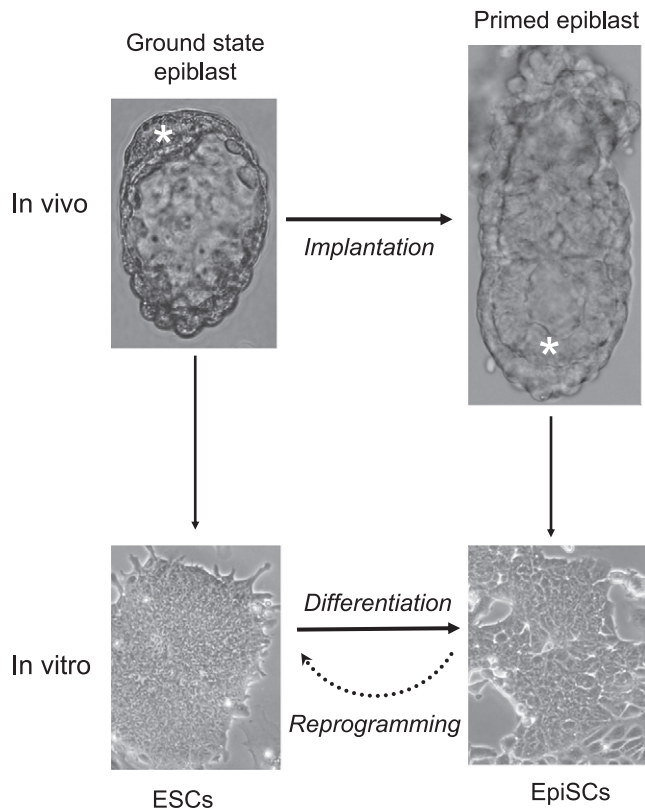
The fertilized mammalian embryo initially undergoes cleavage division in a stereotypic fashion that is unresponsive to extrinsic perturbation. After a fixed number of cell cycles, the individual blastomeres become compacted together, and those on the outside begin to form an epithelium, the trophoblast (Selwood and Johnson, 2006). The resulting structure with an internalized group of cells is called the blastocyst. Up to the time of blastocyst formation, the fate of individual cells can be altered by changing their position within the embryo (Hillman et al., 1972). Transplantation studies confirm that single blastomeres have the potential to generate both extraembryonic and embryonic lineages (Gardner, 1998). Moreover, normal development can ensue following removal of one or more cells from the 8- or 16-cell embryo. This adaptability is exploited in a clinical setting to allow for pregestational diagnosis of severe genetic disease. These findings argue persuasively against an essential

role for asymmetric segregation of determinants inherited from the egg.

Because it can give rise to an entire embryo, the mammalian egg is often described as totipotent. However, this description does not mean that the egg itself has the ability to differentiate into all cell types. In reality, the mammalian zygote follows a determined program of restricted differentiation. If blastomeres are dissociated, they divide and differentiate on schedule to form trophoblast vesicles or microblastocysts with as few as 2 cells (Tarkowski and Wroblewska, 1967; Ziomek and Johnson, 1980). Thus, the egg and blastomeres produce directly only two cell types, the trophoblast and the inner cell mass (ICM). For subsequent development, cells within the ICM must acquire the capacity to generate other cell types and to do so in a flexible manner (Gardner and Beddington, 1988). The ICM produces a second extraembryonic lineage, the hypoblast, and around the same time, the remaining cells develop into pluripotent epiblast. The epiblast is functionally and molecularly distinct from blastomeres and early inner cell mass (Gardner, 1998; Kaji et al., 2007; Kurimoto et al., 2006). Formation of the blastocyst is a deterministic preparatory process dictated by the requirement to elaborate extraembryonic tissues. In comparison with development in other vertebrates, this period may be viewed as a preembryonic stage (Selwood and Johnson, 2006). Thus, rather than representing a diminution in potency from the egg, we suggest that the epiblast constitutes the ground state, meaning a fully unrestricted population that harbors the requisite developmental potency and flexibility to produce all embryonic lineages.

## Regulative Development and Stem Cell Character

The early mammalian embryo has a remarkable ability to accommodate alterations in cell numbers. Additional cells can be introduced into preimplantation embryos and will readily be incorporated into normal development, resulting in chimeric animals. In more extreme examples, minblastocysts, produced by separating 2-cell embryos, or giant blastocysts, generated by combining two or more cleavage stage embryos, develop into normal-sized fetuses and viable animals. This regulative capacity is reminiscent of the flexibility associated with stem cells (Gardner and Beddington, 1988). The finding that progenitors of



**Figure 1. Two Phases of Pluripotency**

Ground state naive pluripotency is established in the epiblast of the mature blastocyst and may be captured in vitro in the form of ESCs. Shortly after implantation, the epiblast transforms into a cup-shaped epithelium and becomes primed for lineage specification and commitment in response to stimuli from the extraembryonic tissues. EpiSCs are the in vitro counterpart of primed epiblast. ESCs can be induced to differentiate into EpiSCs by exposure to activin and Fgf, but the reverse transition requires transfection with Klf4 or other reprogramming factors.

Upper images show mouse embryos at E4.5 and E5.5, or shortly before and shortly after implantation. The white asterisks indicate the epiblast. Note the layer of hypoblast underlying the epiblast in the blastocyst and the proamniotic cavity surrounded by epiblast in the postimplantation embryo. The epiblast is displaced downward after implantation due to proliferation of the trophectoderm-derived extraembryonic ectoderm and the constraint of the uterine wall. Lower images show representative colonies of ESCs and EpiSCs. See [Battle-Morera et al. \(2008\)](#) and [Guo et al. \(2009\)](#) for details of embryo dissection, ESC and EpiSC culture, and photography.

all three blastocyst lineages—trophoblast, hypoblast, and epiblast—can be propagated continuously in vitro ([Rossant, 2008](#)) is consistent with an underlying element of stem cell character. Importantly, however, this plasticity does not extend to transdetermination across lineages. How extraembryonic lineage segregation is secured while pluripotency develops in adjacent cells are intertwined issues in preimplantation development ([Niwa, 2007](#)).

### The Ground State and True Embryonic Stem Cells

The newly formed epiblast is a cluster of 10–20 unspecialized cells sandwiched between the trophoblast and the hypoblast ([Figures 1 and 2](#)). The epiblast generates the entire fetus and single mouse epiblast cells, isolated at this stage and microinjected into another blastocyst, can contribute to all lineages

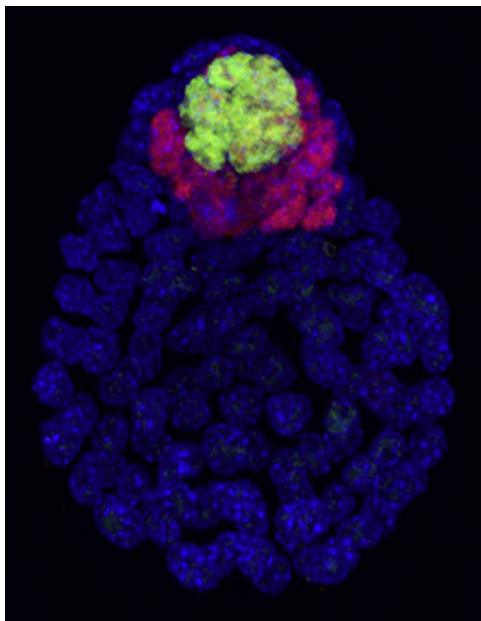
([Gardner, 1998](#)). Functionally, therefore, preimplantation epiblast is the developmental ground state.

Embryonic stem (ES) cells (ESCs) can be derived at this point ([Evans and Kaufman, 1981](#); [Martin, 1981](#)). ESCs represent immortalization of the naive epiblast. Under appropriate conditions, they exhibit unlimited self-renewal capacity while retaining the attributes of preimplantation epiblast identity and potency. Specifically, when returned to the blastocyst, ESCs are readily incorporated into the epiblast and re-enter embryonic development to produce functional soma and germ cells ([Bradley et al., 1984](#)). ESCs also share an epigenetic feature with preimplantation epiblast. This shared trait is the presence of two active X chromosomes in female cells. In female embryos, the paternally inherited X chromosome is silenced during cleavage and remains silent in extraembryonic lineages. Reactivation occurs transiently in the pluripotent lineage prior to implantation ([Heard, 2004](#)). X chromosome activation not only provides an epigenetic signature of ground state pluripotency but, in females, is critical to allow for random inactivation in the soma and, thus, avoid functional hemizygosity.

The epiblast has been identified unequivocally as a source of ESCs by means of microsurgical separation from trophoblast and hypoblast prior to culture ([Battle-Morera et al., 2008](#); [Brook and Gardner, 1997](#)). These experiments also suggested that removal of the extraembryonic tissues may facilitate ESC derivation. ESCs have also been obtained from mouse embryos placed in culture prior to epiblast formation, which has sown some confusion as to their precise origin and identity. It should be borne in mind, however, that embryonic cells are not frozen at the stage when they are put in culture but may continue to follow a developmental program. Thus, cells may progress to the epiblast stage even if isolated from earlier stage embryos. Indeed, mouse ESC lines are molecularly and phenotypically alike, however they have been derived.

The original key to success in deriving ESCs was coculture with mouse embryo fibroblasts, now known to produce the cytokine leukemia inhibitory factor (Lif). Lif activates the transcription factor Stat3, which inhibits ESC differentiation and promotes viability ([Smith, 2001](#)). Genetic and biochemical studies have indicated that stimulation of the Erk pathway by Fgf4 and other extrinsic stimuli is a signal that primes ESCs for lineage specification ([Burdon et al., 1999](#); [Kunath et al., 2007](#); [Stavridis et al., 2007](#)). Recently it has been found that blockade of this pathway and ancillary suppression of glycogen synthase kinase-3 (Gsk3) with selective small molecule inhibitors (3i or 2i) is sufficient to stabilize and sustain ESCs with full pluripotency ([Silva and Smith, 2008](#); [Ying et al., 2008](#)). In our experience, all ESCs, however derived or previously cultured, can be propagated in serum-free culture using these inhibitors with optional addition of Lif. We, therefore, propose that independence from Erk signaling may be a biochemical correlate of the ground state. In line with this idea, use of 2i plus Lif facilitates the isolation of authentic induced pluripotent stem cell (iPSC) lines with full pluripotent capacity ([Guo et al., 2009](#); [Silva et al., 2008](#); [Sridharan et al., 2009](#)). This competence appears to be mediated through a combination of both selection for and stabilization of the ground state ([Silva et al., 2008](#); [Silva and Smith, 2008](#)).

An issue that remains unresolved is whether the derivation of ESCs may entail some reversible epigenetic adaptation to the



**Figure 2. The Ground State Epiblast in the Mouse Blastocyst**  
 Confocal image of a diapause blastocyst immunostained for Nanog (green) and Oct4 (pink), showing that the ICM is partitioned between Nanog-positive epiblast and Nanog-negative hypoblast. Oct4 is present in all ICM cells. The epiblast is entirely surrounded by trophoblast and hypoblast. Blue is DAPI staining of cell nuclei. See *Battle-Morera et al. (2008)* for details of immunostaining and imaging.

ex vivo environment (*Buehr and Smith, 2003*). In other words, are ESCs created in vitro or are they captured directly from the epiblast? By plating dissociated cells, a maximum of three independent ESC clones have been established from a single epiblast (*Brook and Gardner, 1997*). These authors suggest that only a subset of cells within the epiblast may be truly pluripotent and consequently capable of producing ESCs. Relatively low efficiency, and pronounced differences in ability to derive ESCs between mouse strains, could also be due to a limiting epigenetic reprogramming step. Revisiting this experiment using the 3i/2i culture conditions will test the hypothesis of a common ground state both within the preimplantation epiblast and between epiblast and ESCs. We speculate that epiblast cells resident in the blastocyst are in a similar biochemical state to ESCs and may require little or no adaptation to culture if insulated from Erk signaling.

**The Primed Pluripotent State and “Human ESCs”**

After uterine implantation, the rodent epiblast converts into a single-cell layer of columnar epithelium (*Kaufman, 1992*). This conversion is associated with a morphological transformation into a cup-shaped structure known as the egg cylinder (*Figure 1*). In XX embryos, one of the X chromosomes undergoes random inactivation in early egg cylinder epiblast cells (*Heard, 2004*). The epiblast is then subject to a systematic topological bombardment with inductive factors emanating from the adjacent yolk sac and trophoblast tissue (*Beddington and Robertson, 1999*). These molecules include fibroblast growth factors, bone morphogenetic proteins, Wnts, and their respective antagonists. Egg cylinder epiblast cells, therefore, become instruc-

**Table 1. Comparison of Naive and Primed Pluripotent States**

Property	Ground State	Primed State
Embryonic tissue	early epiblast	egg cylinder or embryonic disc
Culture stem cell	rodent ESCs	rodent EpiSCs; primate “ESCs”
Blastocyst chimaeras	yes	no <sup>a</sup>
Teratomas	yes	yes
Differentiation bias	none	variable
Pluripotency factors	Oct4, Nanog, Sox2, Klf2, Klf4	Oct4, Sox2, Nanog
Naive markers <sup>b</sup>	Rex1, NrOb1, Fgf4	absent
Specification markers	absent	Fgf5, T
Response to Lif/Stat3	self-renewal	none
Response to Fgf/Erk	differentiation	self-renewal
Clonogenicity	high	low
XX status	XaXa	XaXi
Response to 2i	self-renewal	differentiation/death

<sup>a</sup>Not applied to primate cells.

<sup>b</sup>Representative examples.

tively specified according to their location. Transplantations between egg cylinders indicate that fates can still be altered at this stage (*Gardner and Beddington, 1988*). However, postimplantation epiblast cells cannot contribute to blastocyst chimeras (*Rossant, 2008*), nor can they give rise to ESCs.

Cell lines have now been derived from postimplantation mouse epiblasts using culture conditions without Lif but including Fgf and activin (*Brons et al., 2007; Tesar et al., 2007*). These cells, termed EpiSCs, express core pluripotency factors, Oct4, Sox2, and Nanog, but differ from ESCs in expression of several other transcripts. EpiSCs are able to differentiate into various cell types in vitro, but this capacity has not been studied extensively to date. It is conceivable that EpiSC lines could be biased in how efficiently they can be committed to different lineages, depending upon their inductive history in the embryo, or defined by the EpiSC culture conditions. If so, this skewing could offer an advantage for directed differentiation along specific lineages. Alternatively, any pre-existing lineage bias may be erased in culture. However, EpiSCs are not competent to contribute to blastocyst chimeras (*Guo et al., 2009; Tesar et al., 2007*) and are, therefore, developmentally and functionally distinct from naive epiblast and ESCs (see *Table 1*).

EpiSCs can also be produced from ESCs in culture (*Guo et al., 2009*). This conversion fulfills the criteria for an authentic differentiation process because the reverse transition has not been observed without genetic manipulation. Consistent with a true differentiation event, one copy of the X chromosome in XX cells is epigenetically silenced as ESCs become EpiSCs. However, EpiSCs still express the canonical pluripotency factors and can be reprogrammed to naive pluripotency by transfection with just a single factor, Klf4 (*Guo et al., 2009*). The resulting iPSCs show reactivation of the X chromosome, exhibit the ESC-specific transcriptional profile, produce high contribution somatic chimeras, and give germline transmission.

The production of mouse EpiSCs from somatic cells by molecular reprogramming has not been reported. However, it would be

difficult to discriminate induced EpiSCs from ground state cells using morphology or the standard Oct4-GFP reporters. It would be interesting to know if reprogramming in Fgf and activin culture conditions yields EpiSCs, particularly if Klf4 was excluded or selectively silenced.

In the absence of chimeras, the standard assay to assess pluripotency is formation of teratomas, which are tumors that contain tissues representative of all three germ layers. Teratomas occur naturally as germline tumors. It is surely not a coincidence that germ cells can be reprogrammed to pluripotency in culture without genetic manipulation (Kanatsu-Shinohara et al., 2004; Matsui et al., 1992; Resnick et al., 1992). Before discovery of ESCs, undifferentiated cells isolated from teratomas were found to retain the capacity at single-cell level to reform multidifferentiated tumors (Kleinsmith and Pierce, 1964; Martin, 1980). These cancer stem cells are called embryonal carcinoma (EC) cells. A few EC cell lines were able to contribute to chimeras, but the majority could not, an outcome traditionally attributed to karyotypic abnormalities (Martin, 1980). It is possible, however, that many EC cells are, in fact, more like EpiSCs than ESCs. It would be interesting to attempt propagation of EC cells from teratocarcinomas generated from ESCs and EpiSCs, comparing ground state ESC conditions with EpiSC conditions. The results of this investigation could reveal whether progression through a tumor induces conversion of the grafted cells in either direction.

A variation of the EpiSC culture regime applied to mouse blastocysts has recently been reported to yield another type of cell line, termed FAB-SCs (Chou et al., 2008). These cultures propagate continuously but are unable to contribute to chimeras or to form teratomas. However, transfer of early passage cultures to ESC medium containing serum factors and LIF results in the ability to produce teratomas and, at very low frequency, chimeras. The authors interpret this observation as reflective of a developmental transition and argue that there may be multiple pluripotent states (Chou et al., 2008). This hypothesis is an intriguing notion, even if it is difficult to comprehend why a cell type with no evident differentiation potential should be described as pluripotent. An embryonic stage to which FAB-SCs would be counterpart is unclear. Critically, the authors do not show data to exclude the likelihood that the FAB-SC cultures harbor a small fraction of epiblast/ESCs that can expand if stimulated with LIF. It is essential to test this possibility because it is well known that residual ESCs can persist in many coculture environments, including EpiSC culture conditions (Guo et al., 2009).

Pluripotent cell lines have been derived from human blastocysts (Thomson et al., 1998). These lines differ significantly from mouse ESCs in their culture requirements, morphology, clonogenicity, differentiation behavior, and molecular profile. Furthermore, the definitive functional criterion for ESC identity, contribution to blastocyst chimeras, cannot be applied in human. Their designation has been based on teratoma formation, which is now shown to be a common property of ESCs and EpiSCs. It has perhaps been unhelpful to use the descriptor "human ESCs" for cells that are so different from the well-defined mouse paradigm. Indeed, it is now argued that these human cells are analogous to rodent EpiSCs (Brons et al., 2007; Rossant, 2008; Tesar et al., 2007). Yet, EpiSCs are obtained from postimplantation epiblasts, whereas human

"ESCs" are derived from cultured blastocysts. As argued above, embryo cells may continue to progress in culture such that human blastocyst cells may reach postimplantation epiblast status from which EpiSCs can be derived. But why should this transition occur in primate embryo cultures when rodent embryos produce ESCs rather than EpiSCs?

### Species Restriction to Propagation of Ground State Pluripotency?

Ever since the first ESCs were derived from mouse blastocysts, strenuous efforts have been made to establish pluripotent stem cell lines from other mammals. These attempts have, in general, been unsuccessful. Until recently, true ESCs had only been validated in the mouse. By inspiration or serendipity, strain 129 was among those chosen for early attempts at mouse ESC derivation (Evans and Kaufman, 1981). It transpires that ESCs can be derived from 129 embryos more readily than from any other mouse strain (Gardner and Brook, 1997). Substrains of 129 exhibit an elevated tendency to develop testicular carcinomas, but it remains unclear whether this susceptibility has any connection with the propensity to yield ESCs. Despite a range of protocols, it remained difficult or even impossible to derive stable chimera-competent ESC lines from most mouse strains. The problem may simply be activation of the Erk pathway by endogenous factors such as Fgf4 and by serum components. Serum-free culture with inhibition of Mek and Gsk3 has facilitated derivation of ESCs from a range of mouse strains tested (Ying et al., 2008). Most significantly, application of these ground state culture conditions has broken the species barrier. Rat ESCs capable of chimera contribution and germline transmission have finally been derived (Buehr et al., 2008; Li et al., 2008).

Nonetheless, unless or until the derivation of true ESCs from human or other primate embryos can be verified, the validity of the ground state hypothesis is open to question. A pertinent issue here is that rats and mice share an unusual method of early development. After formation of the blastocyst, other mammalian embryos do not form an egg cylinder, but instead, the epiblast delaminates as a simple flattened structure called the embryonic disc. During formation of the rodent egg cylinder, the epiblast cells must reorganize from a ball of cells into a cup-shaped epithelium surrounded by hypoblast. This process requires directed apoptosis of the internal epiblast cells (Cocouvanis and Martin, 1999). These events may present a hurdle to progression out of naive pluripotency in rodent embryo explants that extends the window for derivation of ESCs. Conversely, in nonrodent embryo cultures, there may be no barrier to progression to primed epiblasts, and the opportunity for capturing the transient ground state may be minimal.

A second consideration that may mitigate against ESC derivation from primates is diapause. This is a state of arrested embryonic development that occurs naturally in rodents and can be induced experimentally by lowering circulating estrogen to prevent the uterus from becoming receptive for implantation. In diapause, embryos arrest synchronously at the late blastocyst stage after segregation of epiblast and hypoblast (Figure 2). Cell division is greatly reduced, indicating that pluripotency is not intrinsically associated with rapid replication. However, some cell turnover does occur. Therefore, the naive epiblast seems to have an intrinsic facility for self-renewal in these

species. Intriguingly, gene deletion studies have revealed an absolute requirement for Lif/Stat3 signaling in the epiblast during diapause (Nichols et al., 2001). This is a facultative situation, however, because the pathway is dispensable for development without diapause. It is conceivable that the facility for epiblast self-renewal in diapause provides the biological foundation for ex vivo propagation of ground state epiblast. If so, ESC derivation may be more problematic in mammals that do not exhibit diapause, in particular if epiblasts in those species are, in consequence, unresponsive to Lif/Stat3.

### Molecular Requirements for Creating the Naive Ground State

While many genes are important for viability of early embryos, two transcriptional regulators are specifically associated with establishing and maintaining the pluripotent compartment. These factors are the homeodomain containing proteins Oct4 and Nanog. Oct4 is a POU domain transcription factor. It is expressed in the ovum prior to fertilisation, and zygotic transcription from the late 2-cell stage results in distribution to all cells during cleavage. After blastocyst formation Oct4 is gradually downregulated in the trophoblast (Palmieri et al., 1994). In the late blastocyst Oct4 protein can be detected in all cells of the epiblast. It is also expressed transiently in the hypoblast, but is absent from all extraembryonic cells after implantation (Palmieri et al., 1994). Thus, expression of Oct4 becomes progressively restricted to the pluripotent compartment. Clearly, however, Oct4 is expressed more broadly than in the epiblast and therefore alone cannot be sufficient to specify those cells that will become pluripotent.

Expression of Nanog is more tightly correlated with the nascent epiblast (Chambers et al., 2003). Nanog protein is not maternally inherited, but first appears in a seemingly random cellular distribution at compaction (Dietrich and Hiragi, 2007). As the blastocyst develops, Nanog becomes confined to the ICM, though apparently not in all cells. Mosaic distribution in the early ICM is suggested to be reciprocal to the distribution of Gata6, a marker that is later specific to the hypoblast (Chazaud et al., 2006). Traditionally it has been considered that epiblast versus hypoblast fates are determined in the late ICM based on internal or surface location, respectively (Gardner, 1983). The observation of early and mosaic expression of Gata6 has inspired an alternative hypothesis (Chazaud et al., 2006). Molecular specification in the early ICM may dictate separation into distinct territories, such that the hypoblast cells sort out to form a cohesive epithelium overlying the epiblast. However, it is also possible that expression of Nanog and Gata6 may fluctuate in the early ICM and not necessarily correlate with any lineage specification. Indeed, chimera experiments show that while single ICM cells have a tendency to contribute to only hypoblast or only epiblast, a fraction of cells do contribute to both (Chazaud et al., 2006). Furthermore, a more recent live-imaging study points to reversibility of Gata6 expression and suggests that at least an element of hypoblast induction is based on cell position (Plusa et al., 2008). By whichever mechanism the initial segregation is established, in the late blastocyst, hypoblast and epiblast are topologically and histologically distinguishable and exhibit coexclusive expression of Gata6 and Nanog, respectively.

Oct4 expression is maintained continuously in the egg cylinder epiblast. In contrast, Nanog is transcriptionally downregulated at implantation (Chambers et al., 2003). Nanog is then re-expressed in the posterior egg cylinder epiblast (Hart et al., 2004). As gastrulation proceeds and the epiblast differentiates, both factors are extinguished in all somatic lineages (Chambers et al., 2003; Mitsui et al., 2003). Expression persists in the primordial germ cells, however (Yamaguchi et al., 2005).

Oct4 is absolutely required to establish the developmental capacity of the ICM (Nichols et al., 1998). Without Oct4, superficially normal blastocysts form with a substantial ICM, but all the cells assigned to the ICM domain eventually differentiate into trophoblast. Thus, neither hypoblast nor epiblast are produced in the absence of Oct4. Attention is generally focused on the requirement for Oct4 to produce pluripotent cells. It is noteworthy that internal cells in Oct4 null blastocysts transiently exhibit features of ICM character before converting into trophoblast (Nichols et al., 1998). This observation might indicate that the trophoblast differentiation is secondary to an inability to differentiate into epiblast and hypoblast. The absolute requirement for Oct4 in ESCs (Niwa et al., 2000) suggests that it should be essential for progression from ICM to epiblast. Conceivably Oct4 might also be required for hypoblast commitment, given its initial expression in that lineage (Palmieri et al., 1994).

Gene deletion studies have shown that, like Oct4, Nanog is dispensable for blastocyst formation but is absolutely required in the ICM (Mitsui et al., 2003). The ICM cells do not all differentiate into trophoblast, however, pointing to a distinctive function for Nanog compared with Oct4, consistent with their different expression profiles. Interestingly, unlike Oct4, Nanog is dispensable once pluripotency has been attained in ESC cultures, although its absence reduces the threshold for differentiation (Chambers et al., 2007). We speculate that Nanog has a unique function in creating the ground state and a secondary role in stabilizing self-renewal during diapause and in ESCs (Silva and Smith, 2008).

### Conclusions and Perspectives

Development from egg to epiblast is a transition from a determined to an uncommitted state. The underlying mechanism for acquiring pluripotency in the mammalian embryo still eludes molecular definition. Elucidation of this process is not only of intrinsic interest but is likely to inform understanding of molecular reprogramming and induced pluripotent and quasipluripotent states. Indeed, the development of ground state culture conditions utilizing small molecule inhibitors of Fgf/Erk and Gsk-3 signaling has enhanced both derivation of ESCs and reprogramming of somatic cells in rodents. A critical question now is whether the ground state of pluripotency is a core feature of mammalian embryogenesis that can allow generic isolation of naive pluripotent stem cells from a range of species, including human.

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