HYPOTHESIS

Formative pluripotency: the executive phase in a developmental continuum

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ABSTRACT

The regulative capability of single cells to give rise to all primary embryonic lineages is termed pluripotency. Observations of fluctuating gene expression and phenotypic heterogeneity in vitro have fostered a conception of pluripotency as an intrinsically metastable and precarious state. However, in the embryo and in defined culture environments the properties of pluripotent cells change in an orderly sequence. Two phases of pluripotency, called naïve and primed, have previously been described. In this Hypothesis article, a third phase, called formative pluripotency, is proposed to exist as part of a developmental continuum between the naïve and primed phases. The formative phase is hypothesised to be enabling for the execution of pluripotency, entailing remodelling of transcriptional, epigenetic, signalling and metabolic networks to constitute multi-lineage competence and responsiveness to specification cues.

KEY WORDS: Embryonic stem cells, Pluripotency, Epiblast, Lineage specification, Developmental potential

Introduction

Pluripotency may be defined as an intrinsic and flexible cellular potential to generate all cell lineages of the mature organism. Recently, pluripotency has been described in two forms: naïve and primed (Hackett and Surani, 2014; Nichols and Smith, 2009). These terms refer to pre- and post-implantation populations in the embryo and their associated in vitro stem cell states. Naïve and primed pluripotent cells are often presented as directly inter-convertible (Fig. 1A), based on observations in vitro of heterogeneity and reprogramming. However, the two-stage model is an over-simplification that omits a pivotal developmental transformation. Pluripotency may be viewed more accurately as a developmental progression through consecutive phases (Fig. 1B). In this article, the hypothesis presented is that between naïve and primed pluripotency, a formative interval is mandatory to acquire competence for multi-lineage induction. There are two corollaries to this hypothesis: first, that naïve pluripotent cells are unprepared to execute lineage decisions and must necessarily undergo a process of maturation; and, second, that primed cells have initiated a response to inductive cues and are already partially specified and fate-biased. Characterisation of the formative phase is posited to be crucial for understanding the conditions for, and mechanisms of, multi-lineage decision-making.

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The ability to derive mouse ESCs declines precipitately in the peri-implantation period (Boroviak et al., 2014; Brook and Gardner, 1997). This is in spite of the fact that the epiblast expands continuously after implantation and will readily give rise to teratocarcinomas and derivative pluripotent embryonal carcinoma cells (Solter et al., 1970; Stevens, 1970). Explants of post-implantation epiblast can give rise to stem cells if cultured in conditions different to those for ESCs, however. Use of fibroblast growth factor (FGF) and activin instead of LIF enabled establishment of a pluripotent cell type named post-implantation epiblast-derived stem cells (EpiSCs) (Brons et al., 2007; Tesar et al., 2007). EpiSCs can be derived from the epiblast between E5.5 and E8.0 (Osorno et al., 2012). They are heterogeneous but converge on a global transcriptome with features of late gastrula-stage epiblast (Kojima et al., 2014; Tsakiridis et al., 2014). EpiSCs do not integrate well into the ICM and therefore fail to produce substantial chimaerism after morula or blastocyst injection. Importantly, however, when grafted into post-implantation epiblast in whole embryo culture, EpiSCs show evidence of incorporation into developing germ layers (Huang et al., 2012; Kojima et al., 2014; Tsakiridis et al., 2014). Furthermore, transgenic expression of Bcl2 enables EpiSCs to survive after injection into the pre-implantation embryos and subsequently to colonise the egg cylinder (Masaki et al., 2016). This and other genetic manipulations enable EpiSC contribution to somatic tissues in chimaeras, although apparently not to the germ line (Ohtsuka et al., 2012).

Thus, it has so far proven possible to pause developmental progression of the rodent epiblast at initial and late phases of pluripotency and to establish two different stem cell states in vitro. The terminology ‘naïve’ and ‘primed’ was introduced to underscore the recognition that pluripotency is not a unitary state (Nichols and Smith, 2009).

**Dissolution of naïve pluripotency precedes lineage priming**

Cells exiting the naïve ESC ground state in vitro show early morphological changes, involving both cell movement and flattening within 20 h in adherent culture (Kalkan et al., 2016 preprint) and the formation of rosette structures in 3D culture (Bedzhov and Zernicka-Goetz, 2014). These events are potentially significant for biomechanical responsiveness and extracellular matrix signalling. In utero, the epiblast undergoes a morphogenetic transformation shortly after implantation. An amorphous cell cluster converts into a cup-shaped monolayer residing on a basement membrane. Continuous dispersion and re-association might be important to avoid premature specification when localised patterning centres begin to form in the extra-embryonic tissues (Beddington and Robertson, 1999). Mixing might also facilitate elimination of unfit cells through cell competition (Sancho et al., 2013). By contrast, a day later at the pre-streak stage cell fate can be mapped reliably from location, meaning that epithelial integrity is consolidated and cell positions are fixed (Lawson et al., 1991; Tam and Zhou, 1996). By that time, the egg cylinder is pseudo-stratified with overt apico-basal polarity. These cell biological changes might be reflected in discriminating features such as tight junction density or Rho kinase activity.

Mouse pre-implantation epiblast and ESCs are characterised by co-expression, along with Oct4 (Pou5f1) and Sox2, of a suite of transcription factors including Klf4, Tfcp2l1, Esrrb, Klf2, Tbx3, Prdm14 and Nanog that are absent from the immediate post-implantation epiblast (Boroviak et al., 2015). Collectively, these transcription factors constitute a flexible control circuitry that sustains ESC self-renewal (Chen et al., 2008; Dunn et al., 2014; Martello and Smith, 2014; Niwa et al., 2009). Dissolution of this circuitry is evident from the onset of ESC differentiation (Kalkan et al., 2016 preprint; Kalkan and Smith, 2014). Efficient clearance of the naïve transcription factors extinguishes ESC self-renewal capacity and enforces loss of ESC identity. Genetic screens have implicated multiple pathways in the clearance process, acting

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**Fig. 1. Dynamic heterogeneity and phased progression models of pluripotency.** (A,B) In the dynamic heterogeneity model of pluripotency (A), naïve and metastable primed cell states co-exist and are interconvertible. Fluctuation between states creates windows of opportunity for commitment. Germine segregation is not well-delineated within this framework. In the phased progression model of pluripotency (B), cells transit sequentially through naïve to formative to primed forms of pluripotency en route to lineage commitment. In the embryo, this process is an orderly continuum. Ex vivo, however, ESCs cultured in serum may comprise all phases simultaneously and the unidirectional developmental order may even be reversed, creating a situation similar to the dynamic heterogeneity model. In both models, culture of mouse ESCs in 2iLIF ground-state conditions constrains pluripotency within the naïve phase. Dashed lines indicate multi-step differentiation, blue shading represents the Oct4-positive pluripotent populations.
transcriptionally and post-transcriptionally (Betschinger et al., 2013; Leeb et al., 2014). As the naïve factors disappear, a reciprocal gain of expression is apparent for Otx2 and Oct6 (Pou3f1) (Kalkan et al., 2016 preprint). These events at the onset of ESC differentiation recapitulate expression dynamics in the late blastocyst whereby Nanog is extinguished before implantation and Otx2 and Oct6 are upregulated throughout the epiblast (Acampora et al., 2016; Chambers et al., 2003). Other factors for which expression is upregulated early in vivo and in vitro include Sox3, Sall2, the growth factor Fgf5, and the de novo methyltransferases Dnmt3a and Dnmt3b (Boroviak et al., 2015; Kalkan et al., 2016 preprint). Upregulation of Dnmts is accompanied by a substantial genome-wide increase in CpG methylation, in vivo and in vitro, to a level intermediate between the pre-implantation ICM or ESCs and the E6.5 epiblast (Auclair et al., 2014; Kalkan et al., 2016 preprint; Seisenberger et al., 2012).

Significantly, the expression of factors considered to denote lineage specification, such as brachyury, Foxa2 or Sox1, is not evident in cells that have newly and irreversibly exited the ESC ground state, but only becomes appreciable at later time points (Kalkan et al., 2016 preprint; Turner et al., 2014; Zhang et al., 2010; Mulas et al., 2016 preprint). This temporal sequence of gene expression also mirrors events in the embryo. Non-neural lineage specification markers only begin to emerge at the pre-streak stage (E6.0-6.25) in local regions of the epiblast (Russ et al., 2000). Around this time, Nanog is re-expressed in the posterior epiblast (Hart et al., 2004), and Otx2, Oct6 and Sox2 subsequently become restricted to the prospective neuroectoderm in the anterior epiblast where Sox1 is upregulated. Thus, during both ESC entry into differentiation in vitro and epiblast progression in utero, there is a substantial interval of 24 h or longer between the loss of naïve pluripotency and the overt manifestation of lineage priming. During this interval, the pluripotent population might be relatively homogenous in identity, although this has yet to be examined in detail.

**Competence for lineage allocation: the requirement for a formative phase**

The observed temporal separation between exit from naïve pluripotency and fate allocation might be essential for the realisation of multi-lineage potential. The central hypothesis of this article is that a formative period is obligatory for remodelling of the genomic and epigenomic blank canvas of the naïve epiblast to constitute a substrate for lineage specification. Reconfiguration entails not only handover to a distinct gene regulatory network but
also increased engagement with epigenetic regulators, rewiring of signalling pathways, and a switch to predominantly glycolytic metabolism (Kalkan et al., 2016 preprint). In addition, partial epithelialisation and increased interaction with the extracellular matrix are expected to modulate signalling. These events together are envisaged to prepare a template for responsiveness to inductive stimuli and execution of lineage decisions. A paradigm for the concept of remodelling pluripotency to prepare for fate allocation is provided by specification of the germ line. The ability to give rise to germ cells in chimaeras is a hallmark property of mouse ESCs (Bradley et al., 1984). However, ESCs themselves are refractory both to transcription factor determinants and to inductive growth factor cues for germ cell fate (Hayashi et al., 2011; Magnúsdóttir et al., 2013). To become responsive they must lose ESC identity and convert over 24–48 h to a population that has been called epiblast-like cells (EpiLCs) (Hayashi et al., 2011). Transcriptome analyses indicate that EpiLCs are distinct from EpiSCs and resemble the pre-gastrulation E5.5–E6.0 epiblast. Epiblasts at this time are highly responsive to primordial germ cell induction (Oinhata et al., 2009), unlike earlier populations. Importantly, EpiLC cultures can also respond to germ cell specification stimuli. Competence for germine induction is thus not a constitutive feature of pluripotency but is a property acquired during developmental progression. It should also be noted that germine competence is lost in mouse EpiSCs and the late epiblast (Hayashi et al., 2011). Importantly, however, competence appears to be a feature of many, if not all, epiblast cells at E5.5–6.0 (Oinhata et al., 2009), even though only a handful will become specified in normal development. A molecular correlate of germine competence is enhancer remodelling and altered transcription factor occupancy at genes that will be re-expressed in primordial germ cells (Murakami et al., 2016).

Germ cell competence is acquired in the interval between naïve and primed pluripotency. The hypothesis of a formative phase postulates that during this interval competence is also installed for somatic lineage specification. A shared requirement for the formative phase would be consistent with the close association between germ cell and somatic specification from pluripotent founders in divergent mammals (Irie et al., 2015; Johnson and Alberio, 2015) and the key requirement for Blimp1 (Pdml1) to repress somatic fates in order to enable germine specification (Hayashi et al., 2007). The formative remodelling of pluripotency is thus proposed to generate a group of equivalent cells that are uniformly equipped to respond to patterning and lineage specification cues. The neural default model argues that neuroectodermal competence is the fate pluripotent cells will adopt if not instructed otherwise (Muñoz-Sanjuán and Brivanlou, 2002). The formative hypothesis can be compatible with this idea and indeed it is noteworthy that transcription factors that might be key to formative pluripotency, such as Otx2, Oct6 and Sox3, subsequently become expressed continuously (Nakamura et al., 2016).

Factors such as Oct6 and Otx2 are upregulated throughout the early post-implantation epiblast but later become restricted to the anterior presumptive neuroectoderm.

In human, a subset of primed cells in vitro are able to produce primordial germ cell-like cells (Irie et al., 2015; Sasaki et al., 2015).

In formative stem cells, global methylation may be more extensive, as seen for ESCs in serum (Ficz et al., 2013; Habibi et al., 2013; Leitch et al., 2013). **Predictions properties.

### Table 1. Properties of mouse cells in different phases of pluripotency

<table>
<thead>
<tr>
<th>Feature</th>
<th>Naïve</th>
<th>Formative</th>
<th>Primed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expression of Oct4</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Expression of naïve pluripotency TFs</td>
<td>Yes</td>
<td>No</td>
<td>No*</td>
</tr>
<tr>
<td>Expression of early post-implantation TFs</td>
<td>No</td>
<td>Yes</td>
<td>Partial</td>
</tr>
<tr>
<td>Expression of non-ectoderm lineage TFs</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>X inactivation</td>
<td>No</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>Germ cell competence</td>
<td>No</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>DNA methylation</td>
<td>Low</td>
<td>Intermediate</td>
<td>High</td>
</tr>
<tr>
<td>Epithelialisation</td>
<td>No</td>
<td>Partial</td>
<td>Yes</td>
</tr>
<tr>
<td>Apicobasal polarity</td>
<td>No</td>
<td>Partial</td>
<td>Yes</td>
</tr>
<tr>
<td>Homogeneity</td>
<td>Yes</td>
<td>Yes**</td>
<td>No</td>
</tr>
<tr>
<td>Mitochondrial respiration</td>
<td>High</td>
<td>Low**</td>
<td>Low</td>
</tr>
<tr>
<td>Dependence on FGF/Erk signalling</td>
<td>No</td>
<td>Low**</td>
<td>High</td>
</tr>
<tr>
<td>Dependence on Nodal/activin signalling</td>
<td>No</td>
<td>Low**</td>
<td>High</td>
</tr>
<tr>
<td>Formation of blastocyst chimaeras</td>
<td>No</td>
<td>Yes**</td>
<td>No</td>
</tr>
<tr>
<td>Formation of post-implantation chimaeras</td>
<td>No</td>
<td>Yes**</td>
<td>Yes</td>
</tr>
<tr>
<td>In vitro stem cell derivation</td>
<td>Yes</td>
<td>Yes**</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*TFs, transcription factors.

**Predictions properties.

*Focal in pre-gastrulation posterior epiblast in the mouse egg cylinder and in EpiSCs. In the homologous epiblast Nanog appears to be expressed continuously (Nakamura et al., 2016).

*Focal factors such as Oct6 and Otx2 are upregulated throughout the early post-implantation epiblast but later become restricted to the anterior presumptive neuroectoderm.

In human, a subset of primed cells in vitro are able to produce primordial germ cell-like cells (Irie et al., 2015; Sasaki et al., 2015).

In vitro

Factors such as Oct6 and Otx2 are upregulated throughout the early post-implantation epiblast but later become restricted to the anterior presumptive neuroectoderm.

In human, a subset of primed cells in vitro are able to produce primordial germ cell-like cells (Irie et al., 2015; Sasaki et al., 2015).

In formative stem cells, global methylation may be more extensive, as seen for ESCs in serum (Ficz et al., 2013; Habibi et al., 2013; Leitch et al., 2013). **Predictions properties.

### Criteria for evaluating formative pluripotency

The formative epiblast is proposed as the launching pad for multi-lineage differentiation. Several properties of formative pluripotency are expected, relative to attributes of the naïve and primed phases (see Table 1). Predictions can be made for the outcomes of forthcoming single-cell transcriptomic and epigenomic characterisation of the early post-implantation mouse epiblast and of ESC differentiation trajectories.

In terms of global gene expression, formative cells should occupy a transcriptional state space intermediate between naïve and primed populations. The repertoire of expressed genes is expected to be similar in all formative cells, although intrinsic noise in gene expression levels might be greater than in naïve cells due to a more permissive chromatin context. Neither naïve factors nor those that specify a given lineage should be substantially expressed. Regionalised gene expression should be minimal and/or inconsequential in the embryo due to cell dispersion. The formative gene regulatory network is expected to have the general pluripotency factors Oct4 and Sox2 at its core. In the mouse, Otx2 is likely to be a key factor because it is rapidly upregulated in vivo and in vitro (Acampora et al., 2013, 2016; Kalkan et al., 2016 preprint) and co-occupies newly commissioned enhancers with Oct4 (Buecker et al., 2014; Yang et al., 2014). Based on their co-incident upregulation in mouse, significant roles may also be anticipated for Oct6 and Sox3, but other key factors remain to be defined. The specific transcriptional regulators are likely to differ between mice and primates, however (Nakamura et al., 2016).

It is anticipated that the transition into formative pluripotency should entail a profound reconstruction of the chromatin landscape (Zylorz et al., 2015). Global increases in both DNA methylation and polycomb-mediated deposition of H3K27me3 compared with naïve cells (Auclair et al., 2014; Buecker et al., 2014; Kalkan et al., 2016 preprint) are expected to be accompanied by an increased number of bivalent promoters – that is, promoters that bear histone modifications associated with both silencing and activation. Notably, these events are all apparent when mouse ESCs are transferred from 2iLIF to serum (Ficz et al., 2013; Habibi et al., 2013; Leitch et al., 2013; Marks et al., 2012). Enhancer commissioning should be widespread along with selective decommissioning (Acampora et al., 2016; Buecker et al., 2014; Factor et al., 2014; Murakami et al., 2016; Yang et al., 2014). Engagement of chromatin remodellers and epigenetic regulators will equip formative cells for lineage priming. It is noteworthy that mutation of several chromatin remodellers impairs ESC lineage
specification in vitro and compromises, or completely disables, gastrulation in vivo (Hu and Wade, 2012).

The generation of formative pluripotency is expected to depend on specific signalling inputs. Candidate pathways from ligand expression in the embryo are mitogen-activated protein kinase Erk1/2 signalling downstream of FGFs and integrins, and Smad2/3 activity downstream of Nodal (Robertson, 2014). However, these signals also drive lineage specification and at high levels can sustain primed EpiSCs and conventional human pluripotent stem cells (PSCs) (Vallier et al., 2005). The formative phase might therefore be characterised by low threshold activation of these pathways combined with absence or inhibition of other inductive inputs, notably Wnt (Tsakiridis et al., 2014).

The essence of the formative pluripotency concept is that it is necessary for a cell to transit through this remodelling phase in order to prepare for the proper segregation of all definitive embryonic lineages. Therefore, during in vitro differentiation a discrete interval should normally be present between loss of naïve characteristics and the emergence of lineage-specific features. Such a window is apparent at the onset of multi-lineage differentiation of mouse ESCs following withdrawal from 2i in adherent culture (Kalkan et al., 2016 preprint; Mulas et al., 2016 preprint) and a similar formative period might be evident during the early development of embryoid bodies (Rathjen et al., 2002; Shen and Leder, 1992) and gastruloids (van den Brink et al., 2014). Transcription factor-enforced differentiation might conceivably over-ride requirements for a formative phase. In such circumstances, however, the stability and fidelity of mature phenotypes might be compromised due to incomplete epigenetic programming. It should also be noted that extra-embryonic endoderm and trophectoderm do not originate from the epiblast during embryo development, and although some reports suggest they may be generated from ESCs in vitro (Morgani et al., 2013), this would not proceed via the formative phase.

During the formative interval, pluripotent cells have lost naïve identity, and are in the process of acquiring full capability in germline and somatic fate options. Indeed, formative phase cells are expected to become sensitised to lineage-inductive cues. Therefore, they should respond more rapidly and uniformly than naïve cells to inductive cues. However, in the formative phase prospective fates may readily be re-directed by alternative stimuli. Only as specification proceeds and cells become primed for individual lineages through expression of key transcription factors will heterogeneity and lineage bias emerge and re-direction to alternative fates become less readily achieved.

Reconciling phased progression with dynamic heterogeneity

Developmental progression through pluripotency may be paused or even reverted by extrinsic conditions. This may occur during regulative compensation in utero (Gardner and Beddington, 1988; Snow and Tam, 1979) and during establishment of PSCs in vitro (Bao et al., 2009; Boroviak et al., 2014). Culture environments may also corrupt developmental trajectories, however. Mouse ESCs cultured traditionally in the presence of foetal calf serum display heterogeneous expression of multiple genes, including both transcription factors, such as Nanog, Esrrb and Klf4, which are functionally relevant to naïve pluripotency, as well as factors such as brachyury and Foxa2, which are associated with lineage specification (Marks et al., 2012; Torres-Padilla and Chambers, 2014). Furthermore, it has been demonstrated that loss of expression of certain naïve pluripotency markers can be reversible in these culture conditions (Chambers et al., 2007; Hayashi et al., 2008; Toyoooka et al., 2008). These observations of dynamic heterogeneity and the co-existence of naïve and primed features in ESC cultures in
This has been achieved both by resetting established human primed pluripotent cells (Takashima et al., 2014; Theunissen et al., 2014), and by direct derivation from dissociated human ICM cells (Guo et al., 2016). Although further investigation is required to determine the optimal culture conditions and the precise relationship between human naïve cells in vivo and in vitro, the present findings lend support to the premise of conserved principles of pluripotency progression in eutherian mammals, although specific functional attributes might vary (Boroviak and Nichols, 2017).

It follows that a phase of formative pluripotency should be identifiable in primates. Unlike rodents, but in common with other mammals, primate embryos do not develop via an egg cylinder. Instead, the epiblast and hypoblast form a bilaminar disc (Boroviak and Nichols, 2017; Rossant, 2015). In primate embryos, this structure persists for several days prior to gastrulation. It has been poorly characterised due to limitations in accessing early post-implantation material. However, a recent landmark study has now provided the first transcriptomic dataset for a non-human primate, the cynomolgus macaque (Nakamura et al., 2016). There are several important observations in this study. Notably, there is a marked difference between pre- and early post-implantation epiblast, as in mouse. Unlike mouse, however, the post-implantation epiblast appears relatively consistent in gene expression for several days extending to early gastrulation. This could indicate that in primates formative-stage cells persist for longer and potentially self-renew. The study also concluded that conventional human PSCs are most closely related to the late post-implantation epiblast in primates, similar to mouse EpiSCs, whereas the candidate human naïve cells (Guo et al., 2016; Takashima et al., 2014; Theunissen et al., 2014) are more similar to the pre-implantation epiblast.

The availability of an in vivo reference from cynomolgus and an in vitro experimental system in the form of human naïve-like PSCs makes it feasible to address not only whether a formative process occurs, but also whether it is necessary for definitive lineage specification in primates. Significantly, current human naïve cells appear recalcitrant to direct entry into differentiation and are first cultured in primed conditions for several days (Guo et al., 2016; Takashima et al., 2014). It will be important to characterise events during this period and determine the optimal conditions for transitioning naïve cells into full lineage competence. Of note, the formative phase is likely to last longer than in rodent embryos, which advance more rapidly to gastrulation (Fig. 2). This extended time window could facilitate analysis and characterisation. Comparative studies in other mammals, such as pig and rabbit, which progress to gastrulation before implantation, will also be instructive.

Interestingly, a hierarchical population structure has been reported for conventional human PSC cultures (Hough et al., 2014). It will be informative to determine whether the primitive cells at the apex of the hierarchy display features of formative pluripotency. It will also be instructive to examine whether the frequency of such cells changes in various culture conditions that have been reported to alter pluripotent stem cell properties. Identifying cell surface markers and/or knock-in fluorescent reporters of the formative phase will be invaluable for such analyses.

A major challenge will be to derive and propagate stem cells representative of formative pluripotency. This will depend on whether a stable attractor state (Enver et al., 2009) exists within the formative phase that can then be captured in vitro. This is by no means a given. Notably, EpiLCs, which are close to, or may include, formative cells, are considered as a transient population (Hayashi et al., 2011). On the other hand, success in pausing progression at the beginning (naïve ESCs) and end (primed EpiSCs) phases of pluripotency provides a strong precedent. Moreover, the persistence of the post-implantation epiblast in the primate embryo (Nakamura et al., 2016) might endow a greater propensity for ex vivo stability and stem cell derivation.

Formative PSCs, if they can be derived, can be expected to have exited the naïve phase but remain lineage neutral. They should have a discrete transcriptional and epigenetic identity embodying the capture of the corresponding formative pluripotency attractor state. Formative PSCs could have advantageous features compared with both naïve and primed stem cell cultures. In human, stability of the embryonic disc epiblast might be reflected in seamless and efficient stem cell derivation under the right conditions. The resultant human formative PSCs might also be genetically and phenotypically more robust than naïve cells, which appear intrinsically less stable in primates than in rodents (Takashima et al., 2014). At the same time, formative PSCs are expected to be more homogeneous than primed cells. They should be directly responsive to inductive cues, in contrast to naïve cells, but potentially with greater consistency and efficiency than primed cells. Finally, current human naïve-like cells are compromised by the erosion of imprints due to demethylation (Pastor et al., 2016). Formative phase cells are expected to have upregulated de novo and maintenance methyltransferase activities and should therefore be less susceptible to loss of imprints. A brief period in naïve pluripotency conditions could be sufficient for major epigenome remodelling whilst preserving imprints. Reprogramming somatic cells to naïve status then rapidly converting to formative PSCs could therefore be an attractive option for obtaining robust and unbiased cultures with imprints maintained. A complementary approach would be to establish formative PSCs directly from early human ICM explant cultures, which form a post-implantation epiblast-like structure (Deglincerti et al., 2016; O’Leary et al., 2012; Shahbazi et al., 2016).

Conclusions

The testable hypothesis of an essential formative phase focusses attention on the mechanisms that confer multi-lineage competence. Naïve and formative pluripotency are globally distinct; the transition between them involves abrupt network dissolution and replacement. By contrast, the progression from formative to primed pluripotency may entail an incremental set of changes and the boundary between these phases might be less distinct. In the embryo, cells transit through pluripotency with high efficiency and fidelity en route to lineage commitment. In the culture environment, however, this sequence could become derailed, contributing to inconsistent, heterogeneous and abortive in vitro differentiation. Elucidation of the developmental programme of transitions within the pluripotent compartment is fundamental to understanding how lineage decisions are enabled and executed. In addition, recapitulating this programme in vitro might enable improved control and quality of pluripotent stem cell differentiation.

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Competing interests

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