Review

Charting Developmental Dissolution of Pluripotency

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http://dx.doi.org/10.1016/j.jmb.2016.12.017
Edited by David Laurent

Abstract

The formation of tissues and organs during metazoan development begs fundamental questions of cellular plasticity: How can the very same genome program have diverse cell types? How do cell identity programs unfold during development in space and time? How can defects in these mechanisms cause disease and also provide opportunities for therapeutic intervention? And ultimately, can developmental programs be exploited for bioengineering tissues and organs? Understanding principle designs of cellular identity and developmental progression is crucial for providing answers. Here, I will discuss how the capture of embryonic pluripotency in murine embryonic stem cells (ESCs) *in vitro* has allowed fundamental insights into the molecular underpinnings of a developmental cell state and how its ordered disassembly during differentiation prepares for lineage specification.

Pluripotent Cell States

Pluripotency, the capacity to generate all somatic cell types, arises at around embryonic day (E) 4.0–4.5 of murine embryogenesis when the inner cell mass (ICM) segregates the primitive endoderm and the epiblast (Fig. 1). The latter is a population of 10–20 cells endowed with the ability to generate all cells of the adult animal and to give rise to embryonic stem cells (ESCs) *in vitro* by exposure to defined culture conditions [1,2]. ESCs self-renew to regenerate clones of themselves and can be propagated indefinitely while retaining the ability to enter development when transplanted into pre-implantation mouse blastocysts, including transmission of their genome through the germline. When released into differentiation, ESCs recapitulate embryonic development *in vitro* and produce derivatives of all germ layers and germ cells.

Upon embryo implantation at around E5.0–5.5, the epiblast transforms from a mass of cells into a cup-shaped epithelium, also called the egg cylinder, which continues to undergo lineage specification and enters gastrulation at around E6.5 [3]. Although the pre- and post-implantation epiblast differs morphologically and molecularly, egg cylinder cells retain pluripotency and can develop into cell types of all germ layers [4]. Post-implantation epiblast cells mostly fail to respond to ESC culture conditions *in vitro* but give rise to epiblast stem cells (EpiSCs) when exposed to distinct growth factors [5,6]. EpiSCs can resume development when engrafted into the post-implantation epiblast [7] and acquire germ layer fates *in vitro*. However, EpiSCs resist primordial germ cell induction [8], suggesting restricted developmental potential.

The different pluripotent states of the E4.5 and E5.5 epiblasts that give rise to ESCs and EpiSCs *in vitro* have been termed “naïve” and “primed”, respectively [9], although the accuracy of these terms seems worthy of discussion [10]. The pre- and post-implantation epiblasts are transient developmental stages and do not feature self-renewal *in vivo*, while various culture conditions are able to maintain stem cells *in vitro* and preserve the ability to contribute to development upon transplantation in mouse embryos (Table 1). Although transplantation experiments are the gold standard for assaying pluripotency *in vivo*, they are rarely quantitative or performed with single stem cells. Additionally, the host environment can program exogenous cells before entry into development [11]. Transplantation

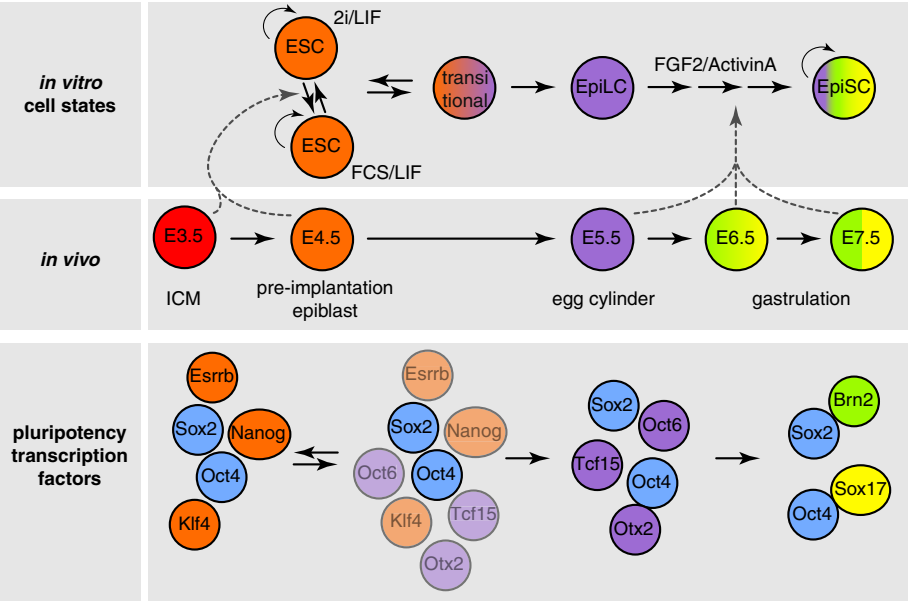

Fig. 1. Alignment of *in vitro* and *in vivo* cell states. Self-renewing ESCs correspond to the E4.5 pre-implantation epiblast, although culture conditions may impose distinct developmental traits. E5.5 egg cylinder pluripotency is recapitulated in EpiLCs that, upon continuous FGF2 and Activin A exposure, give rise to self-renewing EpiSCs in *vitro*. EpiSCs retain developmental properties of the pre-gastrulation and gastrulation embryo (E5.5–E7.5). Alignment of the Oct4/Sox2 network configuration with developmental progression indicates the gradual replacement of naïve-specific TFs (only Esrrb, Nanog, and Klf4 are shown) with post-implantation-specific TFs (Oct6, Otx2, and Tcf15) via a transitional state with ambient expression of both. Eventually, Oct4 and Sox2 irreversibly rewire together with germ lineage-specific transcription factors. Dashed arrows indicate *in vitro* derivation.

is therefore limited in determining the extent of similarity between *in vitro* cultured pluripotent stem cells and their respective *in vivo* counterparts.

Besides testing developmental potential *in vivo*, it is therefore imperative to understand how different stem cell states molecularly and functionally compare to the developing embryo (Fig. 1). ESCs, irrespective of *in vitro* culture conditions, are transcriptionally very similar, although not identical, to the E4.5 epiblast [12–15], suggesting the faithful capture of naïve pre-implantation epiblast pluripotency *in vitro*. In contrast, the relationship between EpiSCs and embryonic development is less clear [8]. Firstly, distinct culture conditions appear to stabilize distinct

Table 1. Culture conditions and transplantation properties of pluripotent cell states *in vitro*

State	Culture condition	Chimera formation	Germline transmission	Reference
ESC (ground state)	KOSR/LIF Chir99021/PD0325901 (2i) 2i/LIF GSK3i/Srci MEKi/TGFβi	yes	yes	[13,26,101,102,112,165,166] [209]
ESC (conventional)	FCS/LIF BMP4/LIF FCS/PKCi	yes	yes	[21,210,211]
EpiLC	FGF2/Activin A/KOSR	nd	nd	[8]
GOF18 GFP pos. EpiSC	FGF2/Activin A	yes	no	[212]
Wnt-off EpiSCs	FGF2/Activin A/(XAV939 or IWP2)	yes	nd	[213,214]
PiCs	FCS/LIF/Proline	yes	nd	[184,186]
F4-EpiSC	FGF2/Activin A/KOSR	yes	no	[215]
INTPSCs	FGF2/Activin A/Chir99021	yes	yes	[216]
EpiSCs	FGF2/Activin A	yes, regionally specified	-	[5–7,17,18,94]
rsEpiSCs	FGF2/IWR1	yes, regionally specified	-	[217]

Note that the EpiLC state is transient and does not self-renew. KOSR (knockout serum replacement); nd (not determined); i (inhibition).