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Contrasting transcriptome landscapes of rabbit pluripotent stem cells *in vitro* and *in vivo*☆

Barbara Schmaltz-Panneau[a], Luc Jouneau[a], Pierre Osteil[c,d,e,f], Yann Tapponnier[c,d,e], Marielle Afanassieff[c,d,e,f], Marco Moroldo[b], Alice Jouneau[a], Nathalie Daniel[a], Catherine Archilla[a], Pierre Savatier[c,d,e,f], Véronique Duranthon[a,*]

[a] *INRA, UMR1198 Biologie du Développement et Reproduction, F-78350 Jouy-en-Josas, France*
[b] *INRA, UMR1313 Génétique Animale et Biologie Intégrative, F-78350 Jouy-en-Josas, France*
[c] *INSERM, U846, Stem Cell and Brain Institute, 18 Avenue du Doyen Jean Lépine, F-69500 Bron, France*
[d] *Stem Cell and Brain Institute, F-69500 Bron, France*
[e] *Université de Lyon, F-69100 Villeurbanne, France*
[f] *INRA, USC1361, F-69500 Bron, France*

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ABSTRACT

Pluripotency refers to the ability for a single cell to differentiate into the three embryonic germ layers. In mice, two types of pluripotent stem cells with different features have been obtained *in vitro*. Naive pluripotent stem cells are derived from the inner cell mass (ICM) of early blastocyst (ESCs) or reprogrammed from somatic cells (iPSCs), while primed pluripotent stem cells are derived from late epiblast (EpiSCs). Cells in a primed pluripotency state are more prone to differentiation and only naive pluripotent stem cells form germline chimera after injection into a blastocyst. Despite numerous attempts, capturing pluripotency in domestic mammalian species has been largely unsuccessful and only primed pluripotent stem cells have been obtained even starting from early blastocyst or reprogramming somatic cells. This raises two questions: whether inner cell mass and epiblast are in naive or primed pluripotency state and what are the transcriptome features of ESCs and iPSCs in these species. To address these questions we compared rabbit ICM, epiblast, ESCs and iPSCs transcriptomes. Our results show that: (i) molecular signature of naïve and primed pluripotency may differ between mice and rabbit embryos; (ii) Genes involved in G1/S transition of the cell-cycle, actin cytoskeleton signaling, development and differentiation pathways are upregulated in ESCs and iPSCs; (iii) ICM and epiblast upregulate pluripotency associated genes and display specific metabolic features. These results denote an advanced primed state of pluripotency for rabbit ESCs and iPSCs and evidence specific functions for ICM and epiblast that are not shared by ESCs and iPSCs.

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* Corresponding author at: Institut National de la Recherche Agronomique, UMR1198 Biologie du Développement et Reproduction, F-78350 Jouy-en-Josas, France. Tel.: +33 134652592.
E-mail addresses: schmaltzbarbara@yahoo.fr (B. Schmaltz-Panneau), veronique.duranthon@jouy.inra.fr (V. Duranthon).

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1. Introduction

Pluripotency refers to the ability for a single cell to differentiate into the three embryonic germ layers, namely ectoderm, mesoderm and endoderm. In mice, pluripotency is an intrinsic property of the inner cell mass (ICM) cells of the blastocyst, which is a small group of self-renewing stem cells. Shortly before implantation, two lineages segregate from the ICM: the primitive ectoderm, often referred to as the early epiblast, which is the founder tissue of the whole embryo proper, and the primitive endoderm, also called the hypoblast, which will later participate in the formation of the yolk sac. Following implantation of the blastocyst in the uterine wall, epiblast cells divide actively while remaining pluripotent, and progressively self-organize into a polarized epithelium referred to as the late epiblast. From gastrulation onwards, late epiblast cells differentiate into the three germ layers and finally loose pluripotency.

In mice, pluripotency can be captured *in vitro* in the form of embryonic stem cells (mESCs) on the one hand, and epiblast stem cells (mEpiSCs) on the other. The mESCs lines are generated from the ICM or early epiblast of the pre-implantation embryo. They exploit LIF/STAT3 and BMP4/Smad signaling to fuel a complex network of transcription factors the so-called core pluripotency network the global activity of which is essential for robust self-renewal (Chen et al., 2008). In contrast, mEpiSCs are generated from the late epiblast of pre-gastrulation embryo. Self-renewal of mEpiSCs depends on FGF2/MEK and activin/Smad signaling pathways, which activate a much less sophisticated and robust network of transcription factors, leading to a higher propensity to spontaneous differentiation (Brons et al., 2007). Moreover, only the mESCs, can colonize the inner cell mass (ICM) of the blastocyst and contribute to the development of all tissue types including germ cells and are thus suitable for generation of knockout mice. It is believed that these differences are largely due to differences in their developmental maturity: mESCs represent a pristine state termed as the naïve state of pluripotency, which characterizes the early epiblast of the pre-implantation embryo. mEpiSCs represent an impaired state, known as the primed state of pluripotency, which characterizes the late epiblast of the early post-implantation embryo (Nichols and Smith, 2009; Zhang et al., 2013).

Pluripotency can also be captured by somatic cell reprogramming with transcription factors (*Oct4*, *Sox2*, *Klf4* and *c-Myc*) in the form of induced pluripotent stem cells (iPSCs). Mouse iPSCs (miPSCs) closely resemble mESCs: they express the cardinal markers of naïve state pluripotency, and they are able to form germline chimeras after injection into blastocysts. Thus the naïve state is thought to represent a default state of pluripotency in rodents.

A number of studies have shown that mESCs and miPSCs on the one hand, mEpiSCs on the other one, differ in many respects (Table 1), epigenetic status and transcriptome being the most obvious. In addition to the core pluripotency network factors *Oct4*, *Nanog* and *Sox2*, mESCs express ancillary factors, the activity of which is essential to naïve pluripotency. mESCs and miPSCs also activate genes expressed in primordial germ cells. In contrast, mEpiSCs do not express the ancillary pluripotency factors aforementioned, and express other factors involved in early commitment to germ layer differentiation (Brons et al., 2007; Tang et al., 2010) (Table 2). This latter observation might explain why mEpiSCs are prone to differentiation. It is worth noting that the transcriptome of mESCs and mEpiSCs differ from that of the ICM and epiblast cells they are derived from (Brons et al., 2007; Borgel et al., 2010; Smith et al., 2012), suggesting that *in vitro* culture impose epigenetic adaptation.

Table 1
Molecular markers of mouse naïve and primed pluripotent stem cells.

	Genes	Authors
Naive state[a]		
Ancillary factors	*Tbx3, Nr0b1, Klf2, Klf4, Klf5, Esrrb, Dppa5*	Tesar et al. (2007); Brons et al. (2007); Nichols and Smith (2009); Tang et al. (2010)
Primordial germ cell markers	*Stella, Dazl, Stra8*	
Other marker	*Rex1*	
Primed state[a] (early commitment gene markers)		
Fgf5, Brachyury, Nestin, Cer1, Sox17, Dkk1, Eomes		

[a] Representative examples.

The situation in non-rodent mammals is markedly different, but rabbit may serve as a model

Despite numerous attempts, capturing pluripotency in domestic mammalian species has been largely unsuccessful and pluripotent stem cells have been derived only from rabbit preimplantation blastocysts (Wang et al., 2007;

Table 2
Functional and molecular features of mouse naïve and primed pluripotent stem cells.

Features	Naïve state	Primed state
Self renewal	Yes	Yes
Morphology of colonies	Dome shaped	Flat monolayered
Clonal expansion	Yes	No
Single cell dissociation after trypsinization	Yes	No
Oct4, Sox2, Nanog expression	Yes	Yes
Functional Oct4 enhancer[a]	Distal	Proximal
Alkaline phosphatase	Yes	No
Cell surface marker SSEA1	Yes	No
Embryoid body formation *in vitro*	Yes	Yes
Teratoma formation after injection in nude mice	Yes	Yes
Germline chimera formation after injection into morula or blastocyst	Yes	No
Chimera formation after injection in epiblast[b]	No	Yes
Metabolism	Oxydative phosphorylation/glycolysis	Glycolysis
Cell cycle: G1 phase	Very short	Short
X chromosome inactivation in female cells	2 active X	1 active X

[a] Okumura-Nakanishi et al. (2005).
[b] Huang et al. (2012).