nature REVIEWS
MOLECULAR CELL BIOLOGY

Stem cell states: naive to primed pluripotency

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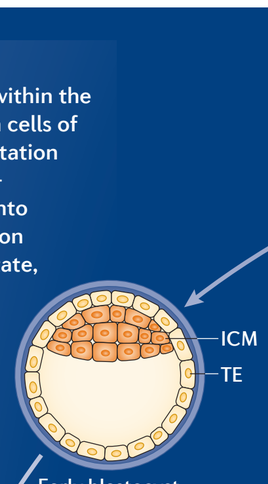
Pluripotency refers to the ability of cells to differentiate into all cell types of the three embryonic germ layers. Deriving and maintaining pluripotent stem cells thus offers the possibility of generating valuable sources of cells for tissue replacement therapies and for developmental studies. Pluripotent cells are found during a short window of time in developing embryos. They progress from a naive ('ground') state to a primed state before lineage commitment. Different culture conditions are being developed to maintain or induce these states *in vitro*.

Pluripotent cells in developing embryos

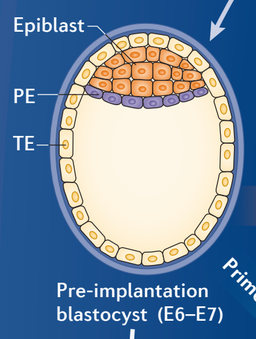
Pluripotency is a transient state *in vivo*. It is acquired within the ICM of developing pre-implantation blastocysts, when cells of the ICM segregate into PE and pluripotent pre-implantation naive epiblasts, and is gradually lost during early post-implantation development, before cells differentiate into somatic lineages. This transition from a pre-implantation pluripotent state to a post-implantation pluripotent state, which are referred to as naive and primed states, respectively, is associated with changes in molecular and functional characteristics.

Differences between human and mouse pre- and post-implantation embryos may be reflected by the different characteristics of naive and primed pluripotent cells *in vitro* and by the different requirements for their maintenance.



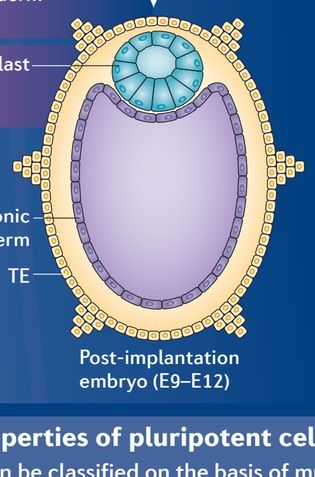
Gene expression in pre-implantation epiblasts

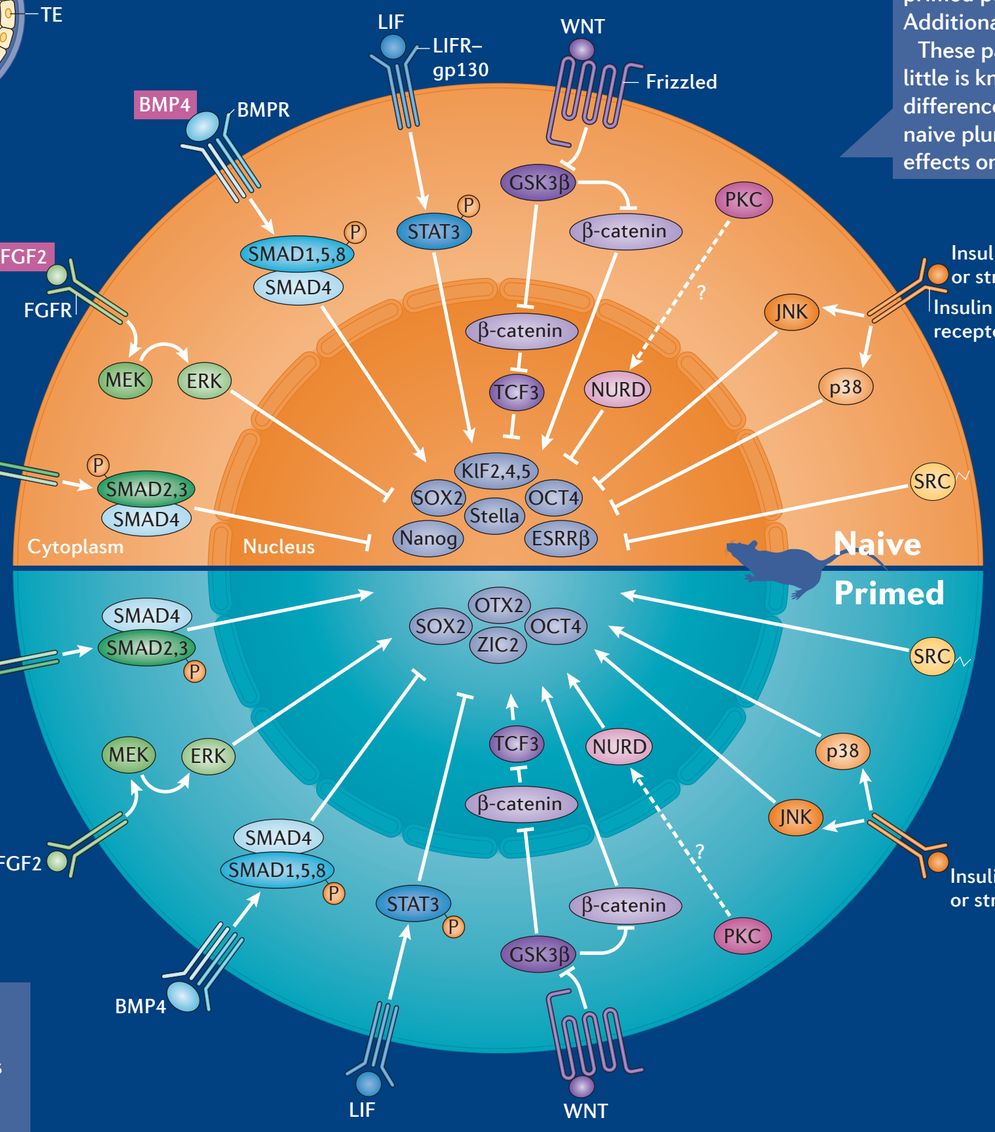
Gene	Human	Mouse
KLF2	No	Yes
KLF17	Yes	No
ERAS	No	Yes
XIST	Low	No
DNMT3L	High	Low



Naive ESCs

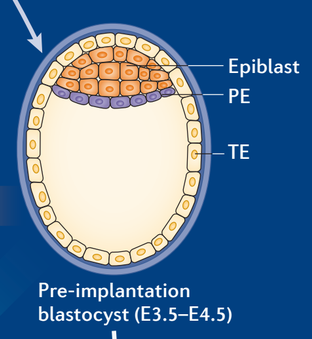
Primed conventional ESCs

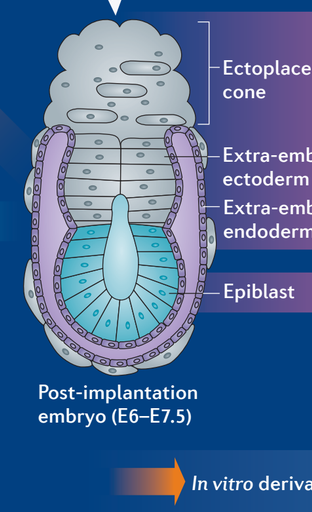




ESCs

EpiSCs





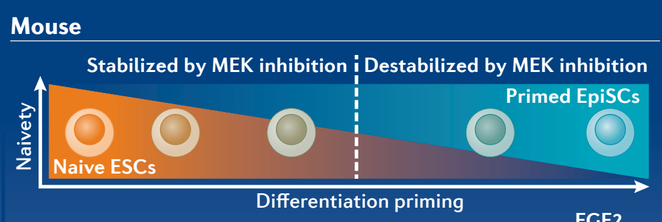
Signalling pathways that affect naive and primed pluripotency in mice

Mouse naive ESCs and primed EpiSCs can be artificially maintained in a self-renewing state *in vitro* by the continuous supplementation of various exogenous cytokines and/or small-molecule inhibitors. These factors regulate signalling pathways that can positively or negatively affect the stability of naive and primed pluripotency, which is regulated by a network of transcription factors. Additional regulatory pathways may yet be revealed.

These pathways affect primed pluripotency similarly in humans and mice, but little is known about their effects on human naive pluripotency. However, some differences have been identified between humans and mice in the regulation of naive pluripotency: low doses of FGF2, activin A and BMP4 may have opposing effects on its stability in different species.

Naive and primed properties of pluripotent cells *in vitro*

Naive and primed states can be classified on the basis of multiple characteristics that each state can retain *in vitro*. Different combinations of exogenous factors confer distinct characteristics to pluripotent stem cells *in vitro*. As a result, cells acquire a distinct set of naive and primed properties. In mice, ESCs cultured in a medium supplemented with 2i (two inhibitors of MEK and GSK3) and LIF, and EpiSCs cultured in a medium containing FGF2 and activin A, constitute the two extremes of the naive and primed pluripotency spectrum; cells maintained in other media are in 'intermediate states' that display a mixture of naive and primed features. Human 'conventional' ESCs, which are considered to be 'primed', are distinct from mouse primed EpiSCs and have various naive features. Optimizing conditions to derive and maintain human naive cells with properties identical to mouse naive pluripotent cells is an ongoing challenge. Moreover, primed cells can be stabilised in a distinct pluripotent state in the presence of FGF2 and WNT inhibitors.



In vitro derivation in naive conditions

In vitro derivation in primed conditions

Potential cross-species differences

Potential link

Pluripotency-associated property	Naive	Primed	Mouse: 2i, LIF	Mouse: FBS, LIF	Mouse: SRCi, GSK3βi	Mouse: MEKi, LIF	Mouse: BMP4, LIF	Mouse: GSK3βi, AXINs	Mouse: FGF2, activin A or FGF2, TGFβ	Rat: 2i, LIF, MEF	Rat: LIF, MEKi, PKCi	Rat: FGF2, activin A or FGF2, TGFβ	Human: OCT4, KLF2, KLF4, 2i, LIF	Human: 2i, LIF, p38i, JNKi, PKCi, ROCKi, FGF2, TGFβ	Human: 2i, LIF, PKCi, MEF (KLF2, NANOG)	Human: 2i, BRAFi, ROCKi, SRCi, LIF, activin A, MEF (FGF2, JNKi)	Human: 2i, BMPi, LIF, MEF	Human: FGF2, TGFβ or FGF2, activin A or FGF2, MEF	Rhesus: 2i, LIF, JNKi, p38i, FGF2, MEF	Rhesus: FGF2, TGFβ or FGF2, activin A or FGF2, MEF
MEK–ERK dependence	No	Yes	●	●	●	●	●	○	○	●	●	○	●	●	●	●	●	○	●	○
Long-term dependence on FGF2 signalling	No	Yes	●	●	●	●	●	●	○	●	●	○		○			○	○	○	○
Long-term dependence on TGFβ–activin A signalling	No	Yes	●	●	●	●	●	●	○	●	●	○		○		○	○	○		○
Dominant OCT4 enhancer	Distal	Proximal	●	●		●		○	○				●	●	●	●		○		
H3K27me3 on developmental regulators	Low	High	●	○					○					●		●	●	○		
Global DNA hypomethylation	Yes	No	●	○					○					● Mild	● Strong			○		○
X chromosome inactivation	No	Yes	●	●		●		○	○				●	●	●	○	○	○	●	○
Dependence on DNMT1, DICER, METTL3, MBD3	No	Yes	●	●					○									○		
Priming markers (OTX2, ZIC2)	↓	↑	●	●		●	○	○	○	○	○	○	●	●	●	●	●	○		○
Pluripotency markers (NANOG, KLFs, ESRRβ)	↑	↓	●	●	●	●	●	○	○	●	●	○	●*	● Mild*	● Strong*	● Strong*	● Mild*	○ *	● Mild*	○ *
TFE3 nuclear localization	High	Low	●	●		●			○					●	●		○	○		
CD24/MHC class 1	Low/low	High/mod	●	●					○					●	●	○		○		
HERV-H and HERV-K expression	High	Low	Primate specific											●	●	●	●	○		
Expressed adhesion molecules	E-cadherin	N-cadherin	●	●	●	●	●	○	○	●	●	○		●	●	●	●	○	●	○
Promotion of pluripotency maintenance via Nanog or Prdm14	Yes	No	●	●	● Nanog only	●	●	○ Nanog only	○					●	●	●	●	○		
Metabolism	OxPhos, glycolytic	Glycolytic	●	●					○							○		○		
Competence as initial starting cells for PGCLC induction	High	Low	●	○					○					●				○		
Capacity of colonization of host pre-implantation ICM and contribution to chimaeras	High	Low	●	●	●	●	●	○	○	●	●	○		●‡	●‡	○‡		○	●‡	○

* No ESRRβ; ‡ Mouse host embryos.

Recommended further reading

Okamoto, I. *et al. Nature* **472**, 370–374 (2011) | Nichols, J. & Smith, A. *Cell Stem Cell* **4**, 487–492 (2009) | Gafni, O. *et al. Nature* **504**, 282–286 (2013) | Marks, H. *et al. Cell* **149**, 590–604 (2012) | Hackett, J. A. *et al. Stem Cell Rep.* **1**, 518– 531 (2013) | Hayashi, K. *et al. Cell* **146**, 519–532 (2011) | Ying, Q.-L. *et al. Nature* **453**, 519–523 (2008) | Tesar, P. J. *et al. Nature* **448**, 196–199 (2007) | Thomson, J. A. *et al. Science* **282**, 1145–1147 (1998) | Rajendran, G. *et al. J. Biol. Chem.* **288**, 24351–24362 (2013) | Wu, J. *et al. Nature* **521**, 316–321 (2015). Please see online supplementary information for a full list of references.

Abbreviations

aPKC, atypical protein kinase C; AXINs, AXIN stabilizer (that is, tankyrase small-molecule inhibitors); BMP, bone morphogenetic protein; BMPR, BMP receptor; DNMT, DNA methyltransferase; E-cadherin; epithelial cadherin; EpiSC, post-implantation epiblast-derived stem cell; ESC, embryonic stem cell; ESRRβ; oestrogen-related receptor-β; FBS, fetal bovine serum; FGF, fibroblast growth factor; gp130, gylcorprotein 130; GSK3β, glycogen synthase kinase 3β; HERV, human endogenous retrovirus; H3K27me3, histone H3 Lys27 trimethylation; ICM, inner cell mass; JNK, JUN amino-terminal kinase; KLF, Krüppel-like factor; LIF, leukaemia inhibitory factor; LIFR, LIF receptor; MBD3, methyl-CpG-binding domain protein 3; MEF, mouse embryonic fibroblast; METTL3, methyltransferase-like 3; MHC, major histocompatibility complex; N-cadherin, neural cadherin; NuRD, nucleosome remodelling and deacetylation; OCT4, octamer-binding protein 4; OTX2, orthodenticle homeobox 2; OxPhos, oxidative phosphorylation; PE, primitive endoderm; PGCLC, primordial germ cell-like cells; PRDM14, PR domain zinc finger protein 14; ROCK, RHO-associated protein kinase; STAT3, signal transducer and activator of transcription 3; TE, trophoectoderm; TFE3, transcription factor E3; TGFβ; transforming growth factor-β; XIST, X-inactive specific transcript; ZIC2, ZIC family member 2.

Acknowledgements

We thank Patrick Tam at the University of Sydney, Australia, and Azim Surani at the Gurdon Institute, University of Cambridge, UK, for their helpful comments.

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Edited by Kim Baumann; copyedited by Harsimran Flora; designed by Vicky Summersby.